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(54) Title: OLIGONUCLEOSIDE COMPOUNDS AND METHODS FOR INHIBITING TUMOR GROWTH, INVASION AND METASTASIS

(57) Abstract

Oligonucleoside compounds useful in inhibiting expression of focal adhesion kinase protein in animals, and related methods and formulations for reducing cancer cell growth, invasion and metastasis. The compounds are selected to be complementary to a target region of a focal adhesion kinase nucleic acid sequence, preferably human FAK mRNA.

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OLIGONUCLEOSIDE COMPOUNDS AND METHODS FOR INHIBITING TUMOR GROWTH, INVASION AND METASTASIS

FIELD OF THE INVENTION

This invention relates to antisense compounds useful in inhibiting the expression of focal adhesion kinase protein in animals and animal cells, and the use of such compounds in suppressing cancer cell growth, invasion and metastasis.

BACKGROUND

The invasion and metastasis of cancer is a complex process which involves changes in cell adhesion properties which allow a transformed cell to invade and migrate through the extracellular matrix (ECM) and acquire anchorage-independent growth properties. Liotta, L.A., et al., Cell 64:327-336 (1991). Some of these changes occur at focal adhesions, which are cell/ECM contact points containing membrane-associated, cytoskeletal, and intracellular signaling molecules. The molecules contained within a focal adhesion include cytoskeletal proteins such as actin, paxillin, and tensin; ECM proteins such as fibronectin, laminin, and vitronectin; cell surface receptors such as the integrins; and protein tyrosine kinases such as *src* family kinases and a recently described tyrosine kinase, the focal adhesion kinase, or FAK.

The FAK gene was originally isolated from chicken and mouse fibroblasts and codes for a unique 125kD cytoplasmic protein tyrosine kinase (p125[FAK]). Schaller, M.D., et al., Proc. Natl. Acad. Sci. USA 89:5192-5196 (1992); Hanks, S.K., et al., Proc. Natl. Acad. Sci. USA 89:8487-8491 (1992). The protein contains highly conserved consensus sequences within its tyrosine kinase domain, but is flanked by long amino-and carboxy-terminal sequences. It also lacks the *src* homology (SH2 and SH3) domains seen in the amino-terminal

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sequences of other cytoplasmic kinases. As FAK has begun to be characterized, a growing body of evidence suggests that FAK is a critical molecule in cell signaling events which regulate cell adhesion and motility, and may be of importance in the invasion and metastasis of cancer. Zachary, I. & Rozengurt, E.,

5   Cell 71:891-894 (1992). First of all, the activity of FAK is directly linked to the *src* oncogene. It has been demonstrated that p125^{FAK} becomes phosphorylated, or activated, in cells which have been transformed with v-*src*, suggesting that FAK may play a role in the transformation by this oncogene. Recent data have shown that p60^{src} stably associates with p125^{FAK}, and it is postulated that the SH2

10   domain of *src* protects FAK from dephosphorylation by phosphatases, resulting in its constitutive activation. Cobb, B.S., et al., Mol. Cell. Biol. 14:147-155 (1994). The linkage of FAK to *src* is particularly intriguing, since levels of c-src activity have been shown to be increased in invasive and metastatic tumors. Weber, T.K., et al., J. Clin. Invest. 90:815-821 (1992); Talamonti, M.S., et al.,

15   J. Clin. Invest. 91:53-60 (1993). This raises the possibility that FAK may be a major downstream mediator of the invasive and metastic process in human tumors.

Another unusual property of FAK which suggests a role in invasion and metastasis is its relationship to the integrins and integrin-mediated signaling

20   pathways. The integrin family of cell surface receptors have been shown to mediate many of the adhesive interactions of tumors and are now thought to be actively involved in signal transduction processes. Juliano, R.L. & Varner, J.A., Curr. Opin. Cell Biol. 5:812-818 (1993). The integrin molecules are composed of noncovalently bound α and β subunits which link the cytoskeleton to the

25   extracellular matrix by binding specific adhesion molecules such as fibronectin, talin, vinculin, and actin filaments. When cellular adhesion is mimicked by clustering integrin receptors with monoclonal antibodies or induced by plating

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cells on a fibronectin-coated substrata, increased phosphorylation of $p125^{FAK}$ has been demonstrated. Kornberg, L., et al., J. Biol. Chem. 267:23439-442 (1992).

Specific integrin expression patterns have been associated with both cellular proliferation and metastasis. For example, overexpression of the $\alpha5\beta1$ integrin in human colon cancer cells has markedly reduced tumorigenicity in nude mice. Varner, J.A., et al., Mol. Biol. Cell 3:232A (1992). In contrast, other integrin expression patterns have been associated with invasion and metastasis, rather than cellular growth. Transfection of the $\alpha2\beta1$ integrin into the RD rhabdomyosarcoma cells has markedly increased tumor metastases in nude mouse tail vein injection assays. Chan, B.M.C., et al., Science 251:1600-1602 (1991). Furthermore, expression of either the $\alpha6\beta4$ laminin receptor or the $\alpha v\beta3$ integrin has been associated with metastatic behavior in studies of melanoma metastases. Ruiz, P., et al., Cell Adhesion Commun. 1:67-81 (1993), Gehlsen, K.R., et al., Clin. Exp. Metastasis 10:111-120 (1992). These findings further raise the possibility of a significant role for FAK in the metastatic process.

The final property of FAK which also suggests a link to cellular growth is its relationship to the growth stimulation of neuropeptides such as bombesin, vasopressin, and endothelin. These molecules exert mitogenic stimuli via receptors which are coupled to effectors via heterotrimeric G proteins. Stimulation of Swiss 3T3 cells with these neuropeptides has led to a rapid increase in specific $p125^{FAK}$ phosphorylation, suggesting that the effector molecules exert their stimuli via FAK. Zachary, I., et al., J. Biol. Chem. 267:19031-34 (1993). Thus, FAK appears to be a convergent pathway for growth stimulatory neuropeptides, transformation by the v-*src* oncogene, and integrin-mediated signaling. Zachary, I. & Rozengurt, E., Cell 71:891-894 (1992).

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SUMMARY OF THE INVENTION

The present invention relates to compositions and methods for inhibiting the growth, invasion and/or metastasis of tumors or cancer cells using antisense oligonucleoside compounds that are complementary to a portion of an FAK mRNA, preferably human FAK mRNA. The inventors have established that such antisense compounds are effective in inhibiting the expression of the FAK protein product in transformed (i.e. cancerous) human cells, and that such inhibition results in reduced cancer cell growth and adhesion, induction of cell apoptosis, reduced cell motility and invasiveness, reduced cell colony formation and anchorage-independent cell growth, and reduced rates of tumor formation.

The FAK antisense oligonucleoside compounds of the invention are chosen to have a length sufficient to bind to and inhibit the expression of the targeted FAK mRNA. The compounds may be of any suitable length, although typically they will have a sequence of from about 6 to about 40, and preferably about 12 to about 30, linked nucleosides. The nucleoside sequence is chosen to be complementary to a selected FAK mRNA target region sequence, such that the antisense compounds are capable of hybridizing to the selected FAK target region of the FAK mRNA within the subject cells and effecting inhibition of FAK expression. The individual nucleosides of the antisense compounds are linked by internucleoside bonding groups ("backbone" linkages) preferably chosen to afford the compounds stability against degradation by endogenous cellular nucleases, and also to enhance stable and specific hybridization to the target FAK mRNA. Such linkages may include natural phosphodiester linkages, but preferably will include one or more nuclease-resistant non-phosphodiester linkages such as phosphorothioate, phosphorodithioate, alkyl- or arylphosphonate, phosphoramidate, phosphotriester, alkyl- or arylphosphonothioate, aminoalkylphosphonate, aminoalkylphosphonothioate, phosphorofluoridate,

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boranophosphate, silyl, formacetal, thioformacetal, morpholino or peptide-based linkages. Specificity and binding affinity toward the target FAK mRNA may be increased through the use of chirally-selected asymmetric linkages, preferably Rp-chiral linkages.

5   The present antisense compounds may be constructed to achieve inhibition of FAK expression by a variety of different mechanisms. For example, the compounds may be designed to form a stable duplex with the RNA so as to block transcription at the ribosome. The duplex blocking mechanism is particularly usefully employed when targeting the 5'-untranslated portion or other

10   non-coding regions of the target mRNA, or elsewhere in the mRNA if ribosomal displacement of the antisense compound does not occur to a significant extent. For target regions where ribosomal displacement is a consideration (e.g., in coding regions), increased duplex stability may be achieved by incorporating a cross-linking moiety in the antisense compound so as to link the hybridized

15   antisense compound to the target mRNA. Alternatively, inhibition of FAK expression may be achieved by using antisense structures which disrupt the integrity or structure of the FAK mRNA molecule, as for example by mRNA cleavage. Cleavage of the target FAK mRNA may be accomplished by choosing antisense sequences capable of activating cellular RNASE H or other endoge-

20   nous cleavage agents, by incorporating a cleavage moiety in the antisense compound, or by co-administering a cleavage substance.

          The FAK antisense oligonucleoside compounds of the invention may also be usefully derivatized or conjugated with, for example, 2'-sugar substituents, particularly electron-withdrawing groups which increase binding affinity;

25   cellular-uptake or membrane-disruption moieties; intercalating agents; radical generators; alkylating agents; detectable labels; chelators; or the like.

          The present invention further includes formulations comprising FAK antisense compounds for use in mammalian cancer therapy, and methods for

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using the same. The antisense compounds of the invention are also useful in the *in vitro* or *ex vivo* study of the biological properties of cancer and other mammalian cells, for example in studies of cell growth, invasion, and metastasis, and studies of the inhibition of such properties.

These and other aspects of the present invention are described in more detail in the following detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-1B show the amino acid sequences for human (HUMFAK), mouse (MUSFAK) and chicken (CHKFAK) focal adhesion kinase proteins, aligned to show sequence homology.

FIG. 2 is a Western blot analysis of p125^{FAK} expression in RD (lane 1), BT20 (lane 2), HT29 (lane 3) and C8161 (lane 4) cell lines.

FIG. 3 is a Western blot analysis showing progressive p125^{FAK} expression in 23 paired colorectal tumors as the tumors invade and metastasize, wherein paired samples from individual patients are indicated by letters at the bottom.

FIG. 4 is a Western blot analysis (top) and a graph (bottom) showing the time course of p125^{FAK} expression in C8161 cells exposed to an FAK antisense compound of the invention and to a missense control.

FIG. 5 is a Western blot analysis showing specific attenuation of p125^{FAK} expression in RD cells treated with FAK antisense compounds FAK1AS (lane 1) and FAK2AS (lane 2), as well as control samples treated with an equivalent concentration of nonsense compound WNT (lane 3) or with 0.3% lipofectin (lane 4), and untreated control cells (lane 5).

FIG. 6A is a bar graph (left) showing loss of cell adhesion in C8161 cells treated with an FAK antisense compound of the invention as compared to control samples treated with a 5bp missense compound (MSN2), and a depiction of stained adherent cells (right) obtained 24 hours after treatment. FIG. 6B is a bar

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graph showing percentage loss of cell adhesion in RD cells treated with AS1, AS2, MSN1, MSN2 oligonucleosides, antisense oligonucleosides to WNT and (G)4 oligonucleoside following a 24 hour treatment period. FIG. 6C is a bar graph showing the relative expression of p125^{FAK} in RD cells treated for 24 hours

5   with AS2, MSN2, WNT and (G)4.

FIG. 7 is a Western blot analysis of p125^{FAK} expression in cells treated with either AS2 or MSN2 oligonucleoside for 24 hours. Subsequently, p125^{FAK} expression was analyzed with AS2 treated cells separated into adherent and nonadherent populations; MSN2 treated cells were treated as a whole population.

10   Lane 1 represents parental cells; Lane 2 represents whole population cells treated with MSN2; and Lanes 3 and 4 represent nonadherent and adherent cells, respectively, treated with AS2.

FIGS. 8A-8C are graphs depicting flow-cytometric analyses of C8161 suspension (8A) and adherent (8B) cells treated with a FAK antisense compound

15   of the invention, or with a 5bp missense compound (8C).

FIGS. 9A and 9B are graphs depicting flow-cytometric analyses of RD cells treated with AS2 oligonucleoside for 24 hours. FIG. 9A is a DNA histogram for nonadherent RD cells treated with AS2; FIG. 9B is a DNA histogram for adherent RD cells treated with AS2. FIG. 9C is a 1.2% agarose gel

20   depicting the degree of DNA fragmentation in adherent (lane 1) and nonadherent (lane 2) RD cells treated with AS2.

FIG. 10 are electron micrographs of nonadherent cells treated with AS2 oligonucleoside, showing nuclear condensation and margination of chromatin (10A), apoptotic bodies containing nuclear fragments with sharply delineated

25   masses of compacted chromatin (10B) and shrinkage of cell size and deeply Giemsa stained nuclei.

FIG. 11 is a micrograph (400x magnification) showing apoptosis in tumor cells treated with AS2 or MSN2 oligonucleoside.

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FIG. 12 is a bar graph (left) showing inhibition of C8161 cell invasive

potential by an antisense compound of the invention compared to a 5bp missense

control, and depictions of stained cell filters (right).

FIG. 13 is a graph of mean tumor volume over time, showing reduced

5   tumor growth in athymic nude mice receiving cancer cells treated with an FAK

antisense compound of the invention as compared to cells treated with a 5bp

missense compound.

DETAILED DESCRIPTION

10   A.   FAK Antisense Compounds of the Invention

FIGURE 1 depicts an amino acid sequence for human FAK (labeled

"HUMFAK", SEQ ID NO:1), as derived from a cDNA clone isolated from the

human breast cancer cell line BT-20. See Example 2 below. This sequence

represents 1052 amino acids of the human FAK sequence. The amino acid

15   sequences for mouse ("MUSFAK", SEQ ID NO:2) and chicken ("CHKFAK",

SEQ ID NO:3) FAK are also shown. The kinase domain of the respective

molecules is boxed. The underlined "recombinant peptide" portion of HUMFAK

corresponds to a 66-amino acid region (198 bp) that was subcloned and

expressed as a fusion product and used for generation of polyclonal antibodies

20   specific for human FAK (see Example 3 below).

FIGURE 1 also shows two regions, labeled "FAK1AS" and "FAK2AS",

which correspond to two FAK mRNA regions targeted for inhibition by

complementary antisense oligonucleosides according to the present invention.

As detailed in Examples 5A-5H below, such antisense compounds were shown

25   to be effective in inhibiting cancer cell growth, cell adhesion, cell invasion,

colony formation and tumor formation, and were effective in inducing cell

apoptosis.

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The FAK antisense compounds of the invention generally include a sequence of nucleosides that is chosen to be complementary to a target region of the target FAK nucleic acid strand, and particularly the human FAK mRNA strand, such that the antisense compound is capable of hybridizing to the target FAK nucleic acid and inhibiting expression thereof. The term "oligonucleoside" refers to a sequence of nucleoside units linked by internucleoside bonding groups ("backbone" linkages), and thus includes oligonucleotides (linked by phosphodiester backbone linkages) as well as nucleoside polymers linked by structures other than phosphodiester bonds. The term "complementary" refers to a sequence of oligonucleosides (or the individual nucleoside units therein), which is capable of forming hydrogen bonds, and thereby base pairing or hybridizing, with the base sequence of a target region of the target FAK nucleic acid to form a Watson-Crick or "double helix" type structure (whether or not actually helicized) or a portion thereof. Complementary sequences include those which have exact base-by-base complementarity to the target region of the target nucleic acid strand, and also includes oligonucleoside sequences which may lack a complement for one or more nucleotides in the target region, but which still have sufficient binding affinity for the target FAK sequence to form a hybridized structure within the subject (e.g., *in vivo* or intracellular) environment, so as to specifically recognize the target sequence and inhibit expression thereof. Complementary sequences also embrace oligonucleoside compounds, or pairs of distinct oligonucleoside compounds, which have sufficient complementarity to achieve triple-strand binding with a target FAK nucleic acid single-strand sequence, or with a double-strand portion of the target nucleic acid such as a hairpin loop structure, thereby to inhibit FAK expression in the subject environment.

The target FAK nucleic acid sequence is preferably FAK mRNA, including FAK pre-mRNA. The particular target region may be chosen from a

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variety of locations in the coding or non-coding portions of the mRNA molecule. Suitable non-coding regions include the 5'-untranslated region, the initiation codon region, the 5'-cap site region, splice acceptor or donor sites, intron branch sites, or polyadenylation regions. Where the target region is a non-coding region, inhibition of protein production can be achieved prior to the translation process by suitable hybridization of the antisense oligonucleoside, and ribosomal displacement of the hybridized oligonucleoside generally does not occur during attempted translation. In such cases translation may be blocked by the effect of complementary hybridization alone, and it will generally not be necessary to incorporate additional inhibition structures (e.g., cross-linking or cleavage moieties) into the antisense compound. Pre-mRNA splicing as a target for antisense oligonucleosides is discussed in R. Kole et al., Advanced Drug Delivery Reviews, 6:271-286 (1991). Where the target region is in the coding portion of the FAK mRNA, it is believed that ribosomal displacement of the antisense compound may sometimes occur during the translation process. In such instances it is useful to incorporate cross-linking, cleavage, RNASE H activating or other expression inhibition structures into the antisense compound in order to increase efficacy. Such structures are described in more detail below. The target region, and the associated sequence of complementary nucleosides in the antisense compound, should be selected such that hybridization is specific to the intended FAK target, thus avoiding or minimizing hybridization with non-FAK nucleic acid sequences in the genome of the subject cell or animal that are not intended to be inhibited. In this regard, publicly-available computer listings of gene sequences may be checked so as to avoid the selection of FAK target sequences similar to known non-FAK genes.

The FAK antisense oligonucleosides of the present invention may be of any suitable length, but preferably are between about 6 to about 40 nucleosides in length, and more preferably between about 12 to about 30 nucleosides. The

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length of a particular antisense compound, the number of complementary bases in the compound, and the identity and location of the complementary bases may be adapted so that suitable target specificity and binding affinity will be achieved under the conditions in which the compound will be used. These conditions include, for example, the effective concentration of the antisense compound inside the cell, the concentration and turnover rate of the target sequence, the desired level of reduction of concentration of the target sequence, the efficacy of expression inhibition, and the mode of inhibition (e.g., catalytic or non-catalytic).

The present FAK antisense compounds preferably are modified to render them resistant to degradation by cellular nucleases or other enzymes that are present *in vivo*. This modification can be accomplished by methods known in the art, e.g., by incorporating one or more internal artificial internucleoside linkages (such as by modifying the phosphodiester linkage to include alternate or additional groups in conjunction with a phosphorus atom, e.g., by replacing one of the non-bridging phosphate oxygens in the linkage with sulfur, methyl or other atoms or groups), and/or by blocking the 3' end of the oligonucleoside with a capping structure. Preferred examples of such nuclease-resistant non-phospho-diester linkages include phosphorothioate, phosphorodithioate, alkyl- (especially methyl-) and arylphosphonate, phosphoramidate, phosphotriester, alkyl- (especially methyl-) and arylphosphonothioate, aminoalkylphosphonate, aminoalkylphosphonothioate, phosphorofluoridate, boranophosphate, silyl, formacetal, thioformacetal, morpholino and peptide-based linkages. Mixtures of such linkages, including mixtures with one or more phosphodiester linkages, are likewise useful and can be utilized to adjust the binding affinity, specificity and expression inhibition characteristics of the subject compounds while maintaining a suitable level of nuclease resistance.

Synthetic methodologies for preparing antisense compounds containing such backbone linkages are known in the art. For example, commercial

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machines, reagents and protocols are available for the synthesis of oligo-
nucleosides having phosphodiester and certain other phosphorus-containing
internucleoside linkages. See, for example, Gait, M.J., Oligonucleotide
Synthesis: A Practical Approach (IRL Press, 1984); Cohen, Jack S., Oligo-
deoxynucleotides Anti-sense Inhibitors of Gene Expression (CRC Press, Boca
Raton FL, 1989); and Oligonucleotides and Analogues: A Practical Approach (F.
Eckstein, 1991); Agrawal, S. (ed.), Protocols for Oligonucleosides and Analogs,
Methods in Molecular Biology, Vol. 20 (Humana Press, Totowa N.J. 1993).
Synthetic methods for preparing methylphosphonate oligonucleosides are
described in Agrawal, above, Chapter 7, pages 143-164 (Hogrefe, R.I.), and in
PCT Application Nos. WO 92/07864 and WO 92/07882. Preparation of
oligonucleosides having various non-phosphorus-containing internucleoside
linkages (such as morpholino, formacetal and peptide nucleic acid (PNA)
linkages and the like) is described in, for example, United States Patent No.
5,142,047 and in PCT Publication Nos. WO 92/02532 (Reynolds, M.A., et al.)
and WO 93/13121 (Cook, P.D.). The disclosures of these synthetic methodology
references are incorporated herein by reference.

Where it is desired to use an antisense compound that is capable of
activating RNASE H for cleavage of the target FAK nucleic acid, a number of
other structural considerations come into play. First, it has been reported that
uncharged backbone linkages are incapable of activating RNASE H. As a result,
such antisense compounds should include an RNASE H activating portion
comprising at least about three consecutive charged (anionic) internucleoside
linkages, as for example phosphodiester, phosphorothioate or phosphorodithioate
linkages or mixtures thereof. Second, it has been reported that various 2'-sugar
substituents (particularly electron-withdrawing groups such as 2'-O-alkyl or 2'-
fluoro) will render the substituted portion of the antisense strand non-activating
to RNASE H, even though binding affinity toward the target nucleic acid is

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increased. Inoue, H., et al., FEBS Letters 215:327-330 (1987); Monia, B.P., et al., J. Biol. Chem. 268:14514-522 (1993). Accordingly, the charged-backbone RNASE H activating portion of such compounds should be 2'-unsubstituted, although 2'-substituents may usefully be employed in other (particularly terminal) non-activating portions of the compound to increase binding affinity. Third, in order to increase nuclease resistance in such antisense compounds, it is preferred to incorporate non-phosphodiester backbone linkages, as for example methylphosphonate, phosphorothioate or phosphorodithioate linkages or mixtures thereof, into one or more non-RNASE H-activating regions of the compounds. Such non-activating regions may additionally include 2'-substituents as discussed above, and, as discussed below, may include chirally-selected backbone linkages in order to increase binding affinity and duplex stability.

Other functional groups may also be joined to the oligonucleoside sequence to instill a variety of desirable properties, such as to enhance uptake of the oligonucleoside sequence through cellular membranes, to enhance stability or to enhance the formation of hybrids with the target nucleic acid, or to promote cross-linking with the target (as with a psoralen photo-cross-linking substituent). See, for example, PCT Publication No. WO 92/02532. Examples of cellular-uptake or membrane-disruption moieties include polyamines, e.g. spermidine or spermine groups, or polylysines; lipids and lipophilic groups; polymyxin or polymyxin-derived peptides; octapeptin; membrane pore-forming peptides; ionophores; protamine; aminoglycosides; polyenes; and the like. Other potentially useful functional groups include intercalating agents; radical generators; alkylating agents; detectable labels; chelators; or the like.

Where it is desired to effect cleavage of the target FAK nucleic acid strand with the antisense compound, a suitable cleavage moiety may be incorporated into the compound. Such cleavage moieties preferably include functional groups

selected to achieve one or more of the functions associated with enzymatic cleavage of RNA. These functions include (1) providing a nucleophilic moiety for attack on the target phosphorus atom, especially by deprotonation of the 2'-OH hydrogen of a target sugar in the target region of the FAK RNA (as achieved, for example, by increasing the local pH about the target sugar and/or by providing a basic or nucleophilic moiety in the vicinity of the target sugar); (2) supplying a proton or other electrophilic moiety for interaction with a phosphorus-bonded lone oxygen atom of the target RNA to form, for example, a protonated phosphate diester (as achieved, for example, by operation of an acidic or electrophilic moiety of the cleavage compound); (3) stabilizing the cleavage transition state, i.e., providing a structure on the cleavage compound to stabilize the intermediate structure or structures assumed by the target RNA during the cleavage mechanism, as by the inclusion of an acid-base moiety and/or other moieties which afford charge neutralization or hydrogen bonding stabilization to the intermediate (particularly polyfunctional groups capable of stabilizing a dianionic phosphorane in a trigonal bipyramidal configuration); and (4) providing a structure to protonate the leaving 5'-O oxygen atom of the target site, as by operation of an acidic moiety of the cleavage compound. See generally Jubian, et al., J. Am. Chem. Soc. 114:1120-1121 (1992), which is incorporated by reference. Preferably, the cleavage moiety comprises two or more distinct functional groups selected to provide two or more of the functions of proton donation, proton acceptance, hydrogen bonding and charge neutraliza-tion. Among these are cleavage moieties comprising two or more amino groups, and wherein at least one amino group is substantially protonated, and at least one amino group is substantially nonprotonated, at physiological pH. Additionally, or alternatively, the cleavage compounds may include a strong Lewis acid moiety, as for example a chelated metal species, which activates the phosphorus-oxygen center of a target phosphodiester bond (or of a target pyrophosphate

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linkage in the case of a 5'-cap region of a target RNA sequence) for direct hydrolytic cleavage by *in situ* water or hydroxide ion. In addition, such antisense cleavage compounds will preferably include a substituent or portion that facilitates rotation of a target RNA sugar portion about the phosphodiester backbone of the target RNA, preferably to position a 2'-OH group of the target RNA for in-line, intramolecular attack on a neighboring phosphorus atom of the target backbone (as achieved, for example, by incorporating an intercalating moiety, a base-omission mismatch, or some other non-complementary structure within the cleavage compound).

Oligonucleosides having one or more chirally pure internucleosidyl linkages (particularly Rp-chiral linkages) may be used and may be preferred in order to increase binding affinity between the subject antisense compounds and the target FAK nucleic acid sequence. Such oligonucleosides, for example with methylphosphonate or phosphorothioate linkages, may be prepared using methods as those described in Lesnikowski, et al., Nucleic Acids Research 18(8):2109-2115 (1990), Stec, et al., Nucleic Acids Research 19(21):5883-5888 (1991), Cook, U.S. Patent No. 5,212,295, or PCT Publication No. WO 93/08296 (Hoke, G.D. & Cook, P.D.). These references are likewise incorporated by reference herein.

The FAK antisense compounds for use in the instant invention may be administered singly, or tandem or separate combinations of the compounds may be administered for adjacent or non-neighboring targets or for combined effects of anti-sense mechanisms in accordance with the foregoing general mechanisms. For example, two separate tandem antisense compounds having complementarity to neighboring target subregions in the FAK nucleic acid strand may be used, where one of the tandem compounds provides a cleavage moiety and the other tandem compound provides a non-complementary structure as described above. Alternatively, each of the two tandem compounds may provide some portion of

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an RNASE H activating region, or some portion of a cleavage moiety, whereby the two compounds act cooperatively following hybridization to adjacent regions in the target strand to effect cleavage or other inhibition of expression of the target strand. Such tandem compounds would be expected to provide greater target specificity (and decreased inhibition of unintended nucleic acid sequences) inasmuch as separate hybridization of two separate antisense compounds is required to achieve inhibition.

B.   Methods and Therapeutic Compositions

When used in mammalian therapy, the FAK antisense compounds may be administered in any convenient vehicle that is physiologically acceptable. The compounds can be formulated for a variety of modes of administration, including systemic, topical or localized administration. Techniques and formulations generally may be found in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA, latest edition. In each case, a therapeutically effective amount of the antisense compound is administered in order to prevent or inhibit the translation of the target FAK nucleic acid. The antisense compound is generally combined with a carrier such as a diluent or excipient which may include fillers, extenders, binding, wetting agents, disintegrants, surface-active agents, or lubricants, depending on the nature of the mode of administration and dosage forms. Typical dosage forms include tablets, powders, liquid preparations including suspensions, emulsions and solutions, granules, capsules and suppositories, as well as liquid preparations for injections.

In the pharmaceutical formulation the antisense compound may be contained within a lipid particle or vesicle, such as a liposome or microcrystal, which may be suitable for parenteral administration. The particles may be of any suitable structure, such as unilamellar or plurilamellar, so long as the antisense oligonucleotide is contained therein. Positively charged lipids such as N-[1-(2,3-

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dioleoyloxi)propyl]-N,N,N-trimethyl-ammoniummethylsulfate, or "DOTAP," are particularly preferred for such particles and vesicles. The preparation of such lipid particles is well known. See, e.g., U.S. Patents Nos. 4,880,635 to Janoff et al.; 4,906,477 to Kurono et al.; 4,911,928 to Wallach; 4,917,951 to Wallach; 4,920,016 to Allen et al.; and 4,921,757 to Wheatley et al. Other non-toxic lipid based vehicle components may likewise be utilized to facilitate uptake of the antisense compound by the cell.

For systemic administration, injection may be preferred, including intraarterial, intravenous and intraperitoneal injection (which are especially preferred), as well as intramuscular and subcutaneous injection. For injection, the cleavage compounds of the invention are formulated in liquid solutions, preferably in physiologically compatible buffers such as Hank's solution or Ringer's solution. In addition, the compounds may be formulated in solid form and redissolved or suspended immediately prior to use. Lyophilized forms are also included. In some instances, the compositions may be infused upstream from the site of the cells whose activity is to be modulated. Implantable drug pumps, as for example Infusaid® pumps (Infusaid, Inc.), are useful for delayed-release intraarterial administration.

Systemic administration can also be by transmucosal or transdermal means, or the compounds can be administered orally. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, bile salts and fusidic acid derivatives for transmucosal administration. In addition, detergents may be used to facilitate permeation. Transmucosal administration may be through use of nasal sprays, for example, as well as formulations suitable for administration by inhalation, or suppositories. For oral administration, the oligonucleosides are formulated into conventional

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as well as delayed release oral administration forms such as capsules, tablets, and tonics.

Antisense compounds of the invention may also be administered by introducing into the cell a DNA construct which produces an antisense compound as described herein within the cells. Such a DNA construct typically contains, in operable association with one another, a transcriptional promoter segment operable in the target cell, a DNA segment that encodes the antisense compound, and a transcription termination segment. Such DNA constructs may be provided in a pharmaceutical formulation as described herein. Such DNA constructs are made and used in accordance with known techniques as set forth in M. Inouye, U.S. Patent No. 5,190,931, the disclosure of which is incorporated by reference herein in its entirety.

For topical administration, the oligonucleosides for use in the invention are formulated into ointments, salves, gels, or creams, as is generally known in the art.

The localized concentration or amount administered to an animal subject may be determined empirically and will depend upon the purpose of the administration, the area to be treated, the effectiveness of the composition, and the manner of administration. The localized concentration at the site of the targeted cells will desirably be in the range of about 0.05 to 50 μM, or more particularly 0.2 to 5μM, although higher or lower dosages may be employed as appropriate. In particular, it is contemplated that relatively high dosage levels may safely be employed in the present context because the FAK gene is overexpressed in cancer cells, and is expressed at relatively low levels in non-cancerous cells. For administration to a subject such as a human, a dosage of from about .01, .1, or 1 mg/kg up to 50, 100, or 150 mg/kg or more may typically be employed.

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The present compounds may also be used in *in vitro*, *ex vivo* or in other non-therapeutic modes in order to study the biological properties of the FAK gene and protein, and their role in normal or cancer cell development, propagation, migration and the like. The present invention is also useful *in vitro* in tissue culture and fermentation techniques where it is desired to inhibit or reduce cell adhesion to facilitate growth of the cells, subsequent processing of the cells, production of proteins or other compounds from the cells, etc. Other uses of the present invention, and suitable antisense compounds to achieve the goals of the invention, will be apparent to those skilled in the art in view of the present disclosure, including the examples that follow. However, it will be understood that the specific examples herein, and the specific antisense structures described, while useful in appreciating the utility of the invention, are not intended to limit the scope of the invention as claimed hereinafter.

C.   Examples

Example 1. Preliminary Studies -- Isolation of Human FAK Homolog and Measurements of Cellular Expression

In preliminary studies relating to the present invention, homologous tyrosine kinase gene fragments were cloned from human cell lines and primary human tumors using low stringency PCR amplification and degenerate oligonucleotide primers based on catalytic domain consensus sequences common to all tyrosine kinases. Cance, W.G., et al., Int. J. Cancer 54:571-577 (1993) (incorporated by reference herein). Using these methods, a 210bp gene fragment of the human homolog of FAK was isolated from a primary human sarcoma, and was found to be expressed in sarcoma, breast and colon cell lines. Weiner, T.M., et al., Ann. Surg. Oncol. 1:18-27 (1994) (incorporated by reference herein). The expression of FAK in normal, adenomatous, invasive, and metastatic human tissue was also studied. Using Northern analysis, increased levels of FAK were

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found in 1 of 8 adenomatous tissues, in 17 of 20 invasive tumors, and in all 15

metastatic tumors. Paired samples of normal tissue did not express detectable

FAK mRNA. See Table 1. This association of FAK overexpression with

invasion and metastasis was a finding common to both the epithelial and

5   mesenchymal tumors analyzed. Furthermore, upon comparison of the levels of

FAK mRNA in paired samples from colon cancer patients, a progressive increase

in densitometrically indexed FAK mRNA was found in 3 of 4 samples as the

tumor invaded and metastasized. Weiner, T.W., et al., The Lancet 342:1024-

1025 (1993) (incorporated by reference herein). These studies are believed to

10   have established the first translational link of FAK expression to the progression

of human cancer.

TABLE 1.   SUMMARY OF NORTHERN ANALYSES OF FAK IN
            HUMAN TUMORS

15

TISSUE:	NORMAL	BENIGN	PRIMARY	METASTASIS
Colon	0/4	1/6	7/8	7/7
Breast		0/2	9/11	4/4
Other*	0/2		1/1	4/4
Total	0/6(0%)	1/8(12%)	17/20(85%)	15/15(100%)

*Normal muscle (2), Primary Thyroid Carcinoma (1) with paired nodal
metastasis (1), Metastatic Carcinoid (1), Squamous Cell Carcinoma (1) and
Melanoma (1)

25

Example 2.   Identification and Characterization of a Human FAK cDNA Clone

The 210bp FAK gene fragment described above was used as a probe to

isolate larger cDNA clones. A cDNA library was first constructed from the BT-

30   20 human breast cancer cell line. Poly-A+ RNA was isolated from BT20 cells,

and first strand synthesis was carried out using a poly-T primer and Maloney-

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Murine Leukemia Virus Reverse Transcriptase. Synthesis of the second strand was performed using DNA Polymerase I, followed by ligation of Not1 linker adapters, Not1 restriction endonuclease digestion, and ligation of the cDNA fragments into a Not1 digested cloning vector. A cDNA clone, spanning 1052 amino acids of the predicted sequence, was identified (see FIG. 1, "HUMFAK").

The clone was found to be homologous to both the mouse and chicken FAK sequences and identical to a recently-published human cDNA clone derived from T-cells (Whitney, G.S., et al., DNA Cell Biology 12:823-830 (1993)).

Example 3. Generation of Recombinant FAK Polypeptide and Anti-FAK Polyclonal Antibodies

A 198bp segment of the FAK cDNA clone of the preceding example was subcloned into a pQE expression vector as described in Bujard, H., et al., Methods in Enzymology 155:416-433 (1987). Fusion protein expression was induced by IPTG at 37°C, followed by purification under denaturing conditions on a Ni-NTA resin column. This provided a hexahistidine fusion protein containing an amino-terminal 6kD fragment of the FAK clone. This segment of the FAK protein (see FIG. 1) was selected in order to allow generation of FAK-specific polyclonal antibodies which would not cross-react with the carboxy-terminal 41kD FAK-related non-kinase protein (FRNK, see Schaller, M.D., et al., Mol. Cell. Biol. 13:789-791 (1993)). The purified fusion protein was analyzed by SDS/PAGE, excised from the gel and injected into rabbits to prepare polyvalent sera.

The antisera recognized a 125kD protein by Western blotting against cell lines (C8161, RD, BT20 and BT474) known to overexpress FAK. The rhabdomyosarcoma (RD) cell line was grown in either RPMI-1640 with 10% heat-inactivated fetal calf serum (FCS), penicillin (100 units/ml) and streptomy-cin (100 mg/ml) or Dulbecco's Modified Eagle's Medium (DMEM-H)

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supplemented with 10% fetal bovine serum (FBS, Hyclone). The C8161, BT20
and BT474 cell lines were grown in RPMI-1640, and maintained at 37°C in a 5%
CO_2 incubator. In the case of the BT474 cell line (purchased from ATCC in
Rockville, Maryland), the RPMI-1640 medium was supplemented with 10%

5   FBS, $10\mu l$/ml insulin and 300 μg/ml L-glutamine. Antibody characterization
further included titering studies to a 1/5000 dilution along with blocking
experiments. Antibody reactivity was completely inhibited by the addition of
recombinant 6kD blocking peptide. Blocking was accomplished by preincuba-
tion of the titered antisera with progressive levels of the recombinant antigenic

10   peptide prior to Western analysis until complete attenuation of the 125kD signal.

Example 4.   Protein-level Measurement of FAK Overexpression in Normal and
             Cancerous Tissue and Cell Lines

       The expression of p125^{FAK} was measured in a variety of normal and

15   cancerous human tissue and cell line samples using the anti-FAK antibody
obtained as described above.

       Initially, FAK expression in the RD (embryonal rhabdomyosarcoma),
BT20 (breast adenocarcinoma), HT29 (colon adenocarcinoma), C8161
(melanoma) and other human tumor cell lines was studied. As shown in FIG. 2,

20   the highest levels of expression occurred in the RD cell line, but expression was
also detected in the BT20, HT29 and C8161 lines. In contrast, some of the cell
lines (such as the breast cell line SK-BR-3) expressed low levels of p125^{FAK}.

       The anti-FAK antibody was also used to assess the change in levels of
p125^{FAK} expression in normal, neoplastic, invasive and metaplastic human

25   tissues. In particular, 91 different tissue samples including epithelially-derived
colon and breast cancers, as well as mesodermally-derived sarcomas were
studied by Western blot analysis. Colon samples included normal mucosa only;
benign, non-invasive polyps; invasive polyps; invasive primary cancers and both

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liver and peritoneal metastatic specimens. Breast samples included benign, non-invasive fibroadenomas; normal breast tissue paired with the infiltrating ductal lesions and a lymph node metastasis. Sarcoma samples included normal muscle; benign mesenchymal tumors such as lipomas and leiomyomas; invasive

5   sarcomas including leiomyosarcoma, rhabdomyosarcoma, neurofibrosarcoma, liposarcoma, synovial sarcoma and fibrohistiosarcoma. Other specimens studied included normal liver and hypercellular parathyroid lesions.

By way of example, colon tumor samples were obtained through operative specimens via IRB-approved protocols and banked at the Tissue Procurement

10   Facility of the Lineberger Comprehensive Cancer Center. Protein was extracted from snap-frozen primary tissues. A 1 cm^3 section of tissue was placed in 3 ml of NP-40 lysis buffer (1% Triton X-100, 20mM Tris, pH 7.4, 150 mM NaCl, 5mM EDTA, 1mM Na_3VO_4, 10mg/ml each of aprotinin and leupeptin). The tissue was homogenized in the lysis buffer using a Polytron (Brinkman), then

15   centrifuged for 15 minutes at 4°C in a microcentrifuge. The amount of protein was measured by the BCA protein assay (Pierce, Rockford, IL). Cell lysate containing 30μg of protein was subjected to 10% SDS/PAGE and electroblotted onto a nitrocellulose membrane as described by Towbin, H., et al., Proc. Natl. Acad. Sci. USA 76:4350-4354 (1979). Immunodetection of blotted p125^{FAK} was

20   accomplished using a 1/2000 titer of anti-FAK antibody along with a 1/5000 titer of anti-rabbit IgG horseradish peroxidase conjugate (Amersham) in non-fat milk. The blots were washed several times in 0.1% TBST (20mM Tris pH 7.4, 150mM NaCl, 0.1% Tween20) and visualization was achieved by chemiluminescence using the ECL detection system (Amersham) followed by X-ray film exposure.

25   In FIG. 3, which shows Western blots for paired colon cancer series, the labels read as follows: NC, normal mucosa; CC, primary invasive tumor; LM, liver metastasis; PM, peritoneal metastasis; RD, embryonal rhabdomyosarcoma cell line (positive control).

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These studies demonstrated the existence of progressive up-regulation of FAK from normal to invasive and metastatic phenotypes, consistent with the transcriptional data described above. The results from the 91 tissue samples are summarized in Table 2. In paired normal and neoplastic colon samples there was no FAK expression in 9 normal mucosal specimens compared to overexpression in 6/7 primary invasive tumors and 9/11 metastatic lesions. These results demonstrated progressive increases in p125^{FAK} expression as tumors invade and metastasize. Additionally, five neoplastic, pre-invasive villous adenomas showed high FAK levels, whereas there was no signal in tubular polyp specimens, suggesting FAK overexpression may be an early event as transformed cells become invasive. In a similar measurements of paired breast cancer samples, 9/16 infiltrating ductal lesions demonstrated FAK overexpression with no signal detectable in the matched normal tissue. Finally, analysis of sarcomas, a histologically diverse family of mesenchymal tumors, showed the highest levels of FAK expression in the biologically aggressive, large (>5cm), high grade lesions. In contrast to invasive tumors, hypercellular neoplastic tissues without invasive potential, such as parathyroid adenomas, did not overexpress FAK. Significant levels of p125^{FAK} expression were detected in 3 samples of large, colorectal villous adenomas, perhaps indicating that these tumors were in the process of becoming invasive. It was notable that the only tumor which did not express high levels of p125^{FAK} was a retroperitoneal colorectal cancer recurrence which slowly developed and was resected 4 years after initial colectomy. Thus, these results confirmed our initial observations, linking overexpression of FAK to the invasive and metastatic phenotype, and suggested that more rapidly growing tumors expressed higher levels of p125^{FAK}.

These observations not only demonstrated up-regulation of p125^{FAK} expression as a tumor became invasive and metastatic, but also suggested that

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p125^{FAK} overexpression accompanied signaling pathways toward invasion and metastasis for a variety of tumors of both epithelial and mesenchymal origin.

TABLE 2. FAK EXPRESSION IN HUMAN TUMORS

TISSUE:	NORMAL	NEOPLASTIC NON-INVASIVE	PRIMARY INVASIVE	METASTASIS
Colon	0/9	5*/6	6/7	9/11
Breast	0/16	0/2	9/16	1/1
Sarcoma	0/2	0/5	8/8	2/2
Other**	0/2	0/4	-	-
Total	0/29	5/17	23/31	12/14

*Villous adenomas (>2cm)
**Normal liver (2), Parathyroid adenoma (4)

Example 5.   Effect of Antisense Oligonucleosides in Inhibiting FAK Expression and FAK-Related Biological Activities

Antisense oligodeoxyribonucleotides having complementarity to portions of the human FAK mRNA were synthesized in order to study their efficacy in inhibiting FAK expression in human tumor cells and their effect on the FAK-related biological properties of the cells.   It was shown that the antisense compounds were not only useful in inhibiting FAK expression, but also inhibited tumor cell growth, cellular adhesion properties, cell motility, cell colony formation, and tumor formation. The compounds were also found to induce cell apoptosis.

A.   Synthesis of Oligodeoxyribonucleosides

Two separate regions near the 5'-terminus of the human FAK clone were selected for targeting by antisense oligodeoxyribonucleotides (see FIG. 1).

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Complementary phosphorothioate-linked antisense oligonucleosides having, respectively, 20 and 24 nucleosides were prepared with the following sequences:

FAK1AS: 5'-ACACTTGAAGCATTCCTTATCAAA-3', SEQ ID NO:4

FAK2AS: 5'-ATAATCCAGCTTGAACCAAG-3', SEQ ID NO:5

These sequences have complementarity with the selected target regions of the human FAK mRNA as follows:

```
HUMFAK        ...Phe Asp Lys Glu Cys Phe Lys Cys ...
         5'-...UUU GAU AAG GAA UCG UUC AAG UGU ...-3'

FAK1AS        3'-AAA CTA TTC CTT AGC AAG TTC ACA -5'


HUMFAK        ...Leu Gly Ser Ser Trp Ile Ile ...
         5'-...CUU GGU UCA AGC UCG AUU AUU ...-3'

FAK2AS        3'-GAA CCA AGT TCG ACC TAA TA-5'
```

In addition, control sequences having a 2-base or a 5-base mismatches were prepared as follows (mismatched bases are underlined):

MSN1:   5'-ATAATCGAGCTTCAACCAAG-3', SEQ ID NO:6

MSN2:   5'-ATAATCGACGTTCAAGCAAG-3', SEQ ID NO:7

A "nonsense" control sequence, derived from the mouse *wnt* protooncogene which was not expressed in the cell lines under study, was also prepared for use in certain of the studies described below:

WNT: 5'-AGCCCGAGCAGGTGGGGCTC-3', SEQ ID NO:8

Another control sequence, a 24-mer containing GGGG [(G)4] nucleotide, which has been shown to have aptomeric effects on cell lines with a reduction in cell proliferation, was used in some of the studies described below:

(G)4: 5'-TATGCTGTGCCGGGGTCTTCGGGC-3', SEQ ID NO:9

The specificity of these sequences was confirmed in GeneBank.

The oligonucleosides were synthesized using standard phosphoramidite chemistry, in the course of which the internucleoside linkages were converted to phosphorothioate linkages to prevent cellular degradation by RNases. After

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synthesis, the oligonucleosides were extracted several times with phenol-chloroform, and then ethanol-precipitated and reconstituted in Hanks' balanced salt solution (HBSS) and frozen at -20°C for storage.

5   B.   General Procedures for Application of Antisense Oligonucleosides to Cell Samples

In the following studies, the oligonucleosides were first preincubated to a final concentration of 0.15 μM with 0.3% lipofection reagent (Gibco BRL) in serum-free Opti-Mem medium (Gibco BRL) in order to form a stable lipid-DNA

10   complex for optimizing transfection. Cell samples were typically prepared by seeding approximately 2×10^5 cells in six-well tissue culture plates with 2 ml of medium, and incubating to 60% confluence. As detailed below, the cells studied included melanoma (C8161), embryonal rhabdomyosarcoma (RD), breast adenocarcinoma (BT20) and breast ductal carcinoma (BT474) cells. The growth

15   medium typically comprised 2 ml RPMI-1640 with 10% heat-inactivated fetal calf serum (FCS), penicillin (100 units/ml) and streptomycin (100 μg/ml). Normal human fibroblast (NHF) cell lines were grown in Eagle's MEM supplemented with 10% FCS.

The lipid-DNA solution was applied to the cells under study by gently

20   overlaying a measured portion of the solution onto a 60% confluent monolayer of cells, followed by incubation for a measured time (typically 0-24 hours) at 37°C in a 5% CO_2 incubator. After this time, the antisense oligonucleoside containing medium was typically removed and the suspension cells isolated, washed and resuspended into 2 ml of normal growth medium. As appropriate,

25   the resuspended cells were replaced onto the remaining adherent cell population for further study.

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C.   Inhibition of p125^{FAK} Expression by FAK Antisense Compounds

Equal numbers of cells were cultured in six-well tissue culture plates and exposed to oligonucleoside/lipofectin reagent solution for varying periods (0-24 hours) as described above. The cells were then allowed to recover for 24 hours.

5   Measurement of p125^{FAK} expression was performed by Western blot analysis with the FAK-specific antibody described above following standard protein level analysis (Pierce).

Attenuation of p125^{FAK} expression in C8161 cells began after 12 hours of exposure to antisense compounds FAK1AS and FAK2AS and was completely

10   abrogated by 18 hours, as exemplified in FIG. 4. Under the same conditions, no significant reduction in p125^{FAK} expression occurred in cells treated with the 5-base mismatch sequence MSN2 or with lipofectin alone. There was a significant attenuation of p125^{FAK} expression with the 2-base mismatch sequence MSN1, although not as complete as with the antisense sequences. FAK expression was

15   seen to recover by 4 days after antisense treatment.

Similar attenuation of FAK expression was obtained when RD cells were treated with the antisense compounds. As shown in FIG. 5, both antisense oligonucleosides completely abrogated p125^{FAK} expression, whereas there was only a minimal reduction in FAK expression in the control samples treated with

20   the WNT oligonucleoside or with lipofectin (0.3%) alone. In addition, the total protein concentrations in the RD cells did not change with FAK attenuation, as indicated by simple Coomassie-stained gels of total protein extracts.

The effects of the antisense oligonucleosides appeared to be highly specific for FAK. Since application of certain oligonucleotide sequences is

25   known to result in general inhibition of gene expression, we examined the expression in antisense treated cells of other tyrosine kinases known to associate with FAK. The expression of p60^{src} and p59^{fyn} were not significantly altered in these cells. These results suggested not only that the antisense effects of FAK

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were specific, but also that FAK may function as a downstream element to *src* and *fyn* in these cellular signaling pathways.

D.   Inhibition of Cellular Adhesion by FAK Antisense Compounds

Disruption of FAK signaling pathway with the FAK antisense oligo-nucleosides was shown to cause profound changes in cell phenotype. The most significant change observed was a marked loss of cellular adhesion (see FIG. 6A). In each of the model cell lines (C8161, RD, BT20 and BT474), the antisense oligonucleosides appeared to disrupt the cell-matrix interactions. After exposure to antisense, treated cells showed a tendency to round-up and enter suspension. This cellular morphologic effect was progressive throughout the duration of antisense oligonucleoside exposure and continued beyond the twenty-four hour treatment period.

By way of example, the effect of the antisense compounds FAK1AS and FAK2AS on cellular adhesion properties was measured by exposing C8161 cells to FAK antisense or to the 5bp missense control MSN2 for 24 hours. The numbers of cells adherent to the cell culture plates (solid bar) and cells in suspension (white bar) were counted at 0, 6, 12, 18 and 24 hours after antisense or control oligonucleosides were added (see FIG. 6A). Loss of adherence in the FAK antisense-treated cells began between 12 and 18 hours after oligonucleoside addition, correlating with the loss of $p125^{FAK}$ expression (FIG. 4). This loss of adhesion was also visualized in hematoxylin and eosin-stained cells 24 hours after FAK antisense treatment. Cells treated with the 5bp missense sequence MSN2 retained their normal adherent characteristics in the staining studies. Similar inhibition of cell adhesion was observed with RD and BT20 cells treated with FAK antisense oligonucleosides. Using RD cells, adherence loss after 24 hours was approximately 40% using AS1 and nearly 70% using AS2 antisense oligonucleoside (FIG. 6B). While 60% of the RD cells lost adherence using

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MSN1 oligonucleoside, only about 10% of cells treated with MSN2 lost adherence (see FIG. 6B) following 24 hours of oligonucleoside treatment. To ensure that the phenotypic changes were specifically caused by attenuation of p125^{FAK}, normal human fibroblasts, which did not express high levels of p125 ^{FAK},

5   were treated with AS2 oligonucloside. No significant changes were observed in antisense-treated cells compared to missense-treated cells.

The observed loss of adherence was not specific to sarcoma cells only. When a human epithelial breast cancer cell line (BT474) and a melanoma cell line (C8161) were treated with AS2 oligonucleoside, a marked loss of adherence

10   was also observed in both of these tumor cell lines. Western blot analyses of adherent and nonadherent populations showed that p125^{FAK} was undetectable in nonadherent cells but was not altered in either antisense-treated or missense-treated adherent cells for the RD, C8161 and BT474 cell lines (FIG. 7).

RD cells treated with either of the control oligonucleosides WNT or (G)4

15   did not show significant loss of adherence (FIG. 6B) and expression of p125^{FAK} was unchanged (FIG. 6C). The loss of adherence following treatment with AS2 was found to be specific to AS2, correlating with the attenuation of p125^{FAK} expression.

20   E.   Inhibition of Cell Growth and Inducement of Apoptosis by Attenuation
            of p125^{FAK} Expression

Apoptosis was evaluated by performing analyses of cell viability, flow cytometry, DNA fragmentation, Giemsa staining and electron microscopic morphology on certain cells following 24 hours of oligonucleoside treatment.

25   Viable cells were counted following staining with 0.4% trypan blue. For Giemsa staining, cells were centrifuged onto microscopic slides using a Cytospin 2 centrifuge (Shandon Lipshaw, PA) and stained with a Diff-Quik stain kit (Baxter). Quantitation of the number of apoptotic cells was accomplished by

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counting the number of apoptotic cells versus the number of total cells sighted in ten 400X microscopic fields. For flow cytometric analysis, cells were pelleted, washed in HBSS and fixed with 70% ethanol for 1 hour at 4°C. Cells were then washed in HBSS and resuspended in PI buffer (20 μg/ml propidium

5   iodide, 20μg/ml RNASE in PBS, pH 7.4) at a final concentration of 1×10^6 cells/ml and analyzed using FACScan (Becton-Dickinson).

Analysis of internucleosomal degradation of genomic DNA was performed following oligonucleoside treatment. The cells were collected from each of the separated adherent and nonadherent populations, rinsed with HBSS

10   and lysed in 100μl of lysis buffer [10mM TRIS-HCl (pH 8.0), 10mM EDTA (pH 8.0), 0.5% Triton X-100 (Sigma)]. Lysates were centrifuged at 13,000 × g for 20 minutes and supernatants containing soluble fragmented DNA were collected, treated with RNaseA (100 μg/ml) for 1 hour at 37°C, followed by proteinase K (200μg/ml) treatment in 1% SDS for 2 hours at 50°C. The samples were then

15   extracted twice using phenol-chloroform, once again using chloroform, and then ethanol precipitated with one-tenth volume sodium acetate (pH 5.3) and two volumes ethanol (100%) for 1 hour at -70°C. Electrophoresis of 250 ng DNA was performed on a 1.2% agarose gel in 1 XTAE (0.04 M TRIS-acetate, 1 mM EDTA, pH 8.0) for 1.5 hours at 50V. The gel was then stained in 1 XTAE and

20   0.5 μg/ml ethidium bromide for 15 minutes at room temperature.

Electron microscopic analysis of cell morphology was performed on treated adherent and nonadherent cells. Nonadherent cells were collected, washed with warm serum-free media, and then fixed using 3% glutaraldehyde in medium overnight at 4°C. Samples were rinsed with PBS, embedded in Epon

25   and cured for 3 days. Thin sections (approximately 60-90 nm) were stained with 5% uranylacetate and 2.7% lead citrate and examined using a Zeiss transmission electron microscope.

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Evaluation of nonadherent antisense-treated cells by trypan blue exclusion showed that they were approximately half the size of the adherent cells and exhibited greater than 90% viability, evidencing that the nonadherent cells were not simply necrotic. Flow-cytometric analysis of propidium iodide-stained C8161 suspension cells (20μg/ml propidium iodide in PBS cells, fixed in 70% ethanol) indicated that the DNA content of 60% of the nonadherent population was less than 2n. The cells appeared as a gaussian peak to the left of G_0G_1 which is characteristic of apoptosis (FIG. 8). Furthermore, the nonadherent cells appeared to be arrested in the G1 phase. These flow cytometric findings correlated with the inhibition of cell growth observed following antisense therapy. Furthermore, the C8161 cells showed no significant growth for three days (72 hours) following exposure to antisense oligonucleosides while MSN2 control treatments showed no alterations in transformed cell growth. The effects on C8161 cells specifically correlated with the antisense attenuation of p125^{FAK} expression (FIG. 4). These observations are similar to the anoikis phenomenon described by Frisch, et al. (J. Cell Biology 124:619-626 (1994)) and is a further indication that FAK may play a role in regulating these events.

Similar growth inhibition effects were observed in RD and BT20 cells treated with FAK antisense oligonucleosides. For example, the DNA histogram for nonadherent RD cells, when compared to the adherent group, contained a larger population with low DNA content, indicative of apoptosis (FIG. 9A). Indeed, 60% of these cells had a DNA content of less than 2n, as measured by FACScan analysis. In contrast, the majority of adherent cells treated with antisense oligonucleosides contained a normal DNA content (FIG. 9B).

Because flow cytometric analysis suggested that these antisense treated cells were apoptotic, several other apoptotic criteria were assessed on the nonadherent cells. One of the hallmarks of apoptotic cell death is endonuclease cleavage of genomic DNA into nucleosomal size fragments of 200 bp.

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Following DNA extraction from both the adherent and nonadherent antisense treated cells and from cells treated with missense oligonucleoside, the nonadherent population demonstrated nucleosomal fragmentation. In contrast, the adherent cells which did not have attenuated FAK expression maintained intact

5   DNA (FIG. 9C).

A further distinguishing event in apoptosis is the degradation of the nucleus into vesicles. When several of the nonadherent cells with different morphologies were examined, a few cells appeared to be early in the apoptotic process, with their nuclear envelope still evident but with condensation and

10   margination of the chromatin (FIG. 10A). However, the majority of the cells in the nonadherent population had the morphology demonstrated in FIG. 10B, in which several apoptotic bodies were evident, and the nuclear envelope had dispersed while the plasma membrane remained intact. Giemsa staining of the cells allowed quantitation of the relative number of cells undergoing apoptosis

15   in AS2 treated cells (FIG. 10C).

Following staining of both the adherent and nonadherent cell populations, the percentage of apoptotic cells was determined by counting the number of apoptotic cells versus the total number of cells sighted in ten microscopic fields (400× magnification). Cells treated with MSN2 showed a negligible percentage

20   of apoptotic cells, while cells treated with AS2 had a significantly higher percentage of apoptosis in each of the cell lines studied (see FIG. 11 and Table 3). These results indicate that attenuation of p125^{FAK} causes cells to undergo apoptosis.

The percentage of apoptotic cells in the RD, C8161 and BT474 cell lines

25   following treatment with AS2 and MSN2 controls are summarized in Table 3 below. No measurable apoptosis was observed in any of these cell lines using the 5bp missense control.

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TABLE 3. PERCENT OF APOPTOSIS

Cell Type	Oligonucleoside Type	
	AS2	MSN2
RD	45.5%	0.0%
C8161	34.1%	0.0%
BT474	30.7%	0.0%

F.   Inhibition of Tumor Cell Motility by FAK Antisense Compounds

Tumor cells interact with basement membranes in a manner fundamentally different from normal cells. The results presented above suggested that antisense attenuation of p125^{FAK} expression might interrupt the ability of tumor cells to bind to their adjacent basement membrane, an initial requirement in the sequence of events leading to invasion. A subsequent step in the invasion process involves alterations in cellular motility which allow cells to actually propel themselves across the basement membrane and enter the interstitial stroma. To assess the role of FAK in these events, we used an *in vitro* cell invasion assay and determined the changes in the migration patterns of C8161 cells, which are known to the highly invasive, after attenuation of p125^{FAK} expression.

The invasion assay allowed measurement of the invasive potential of cells through a reconstituted basement membrane in a modified Boyden chamber. Biocoat Matrigel Invasion Chambers (Becton Dickinson) were rehydrated over 2 hours by adding 2 ml of warm Opti-Mem and placed into individual wells of Falcon six-well culture plates. Conditioned medium was obtained by incubating human fibroblasts for 24 hours in Opti-Mem. This medium was used as a source of chemoattractants and was placed in the lower compartment of the Boyden chambers. 2×10^5 C8161 cells pre-treated with FAK antisense or 5bp missense oligonucleosides were suspended in Opti-Mem containing 10% FCS and added

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to the rehydrated upper chambers. Assays were carried out at 37°C in 5% CO_2. At the end of the incubation (about 24 hours), the cells on the upper surface of the filter were completely removed by wiping with a cotton swab under direct microscopic visualization. The filters were fixed in methanol and stained with

5   hematoxylin and eosin. Cells from various areas of the lower surface were counted to correlate cell invasion of the reconstituted basement membrane. Each assay was performed in triplicate.

As shown in FIG. 12, the antisense treated cells demonstrated a dramatically lower invasive potential (5.8 ± 4.3%) compared to the missense

10   treated control (40.6 ± 5.2%). An emerging theme in the study of tumor invasion is that, in addition to unrestrained growth, tumor cells display an imbalanced regulation of motility and proteolysis. These *in vitro* results suggest that FAK may be closely involved in the former of these two critical processes.

15   G.   Inhibition of Anchorage-Independent Growth Properties by FAK
            Antisense Compounds

In addition to motility changes, invasive and metastatic cells develop enhanced anchorage-independent growth properties. This phenomenon is most apparent in human malignancy during the events leading to tumor dissemination

20   in processes such as carcinomatosis, as well as in tumor cell colony formation in metastatic target organs. We assessed the anchorage-independent growth properties in FAK antisense treated cells by measuring their ability to form colonies in soft agar.

Two different tumor cell lines (C8161 and RD) were used in these studies.

25   Oligonucleoside treated cells were seeded at a density of 5 x 10^4 cells per plate in a 0.33% top agarose layer in RPMI-1640 supplemented with 10% fetal bovine serum. The semisolid cell containing agar was layered onto 0.5% hard agar and incubated on scored tissue culture dishes (60 mm in diameter) in a humidified,

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5% CO_2 atmosphere at 37°C. The dishes were fed once every several days with 1.0 ml of 1X medium. Colony formation efficiency was determined after two weeks in triplicate, blinded fashion by phase contrast microscopy counting all colonies larger than 70mm in diameter.

5   The results of these studies are shown in Table 4, wherein the values represent the number of colonies in soft agar along with a calculated colony forming efficiency (total colonies per plate/total cells per plate, times 100). Following specific attenuation of FAK expression using either the FAK1AS or FAK2AS antisense compounds, a marked reduction in colony formation in soft

10   agar was seen compared to cells treated with the control missense oligo-nucleoside MSN2. After two weeks of culture there was a 81% reduction in colony formation efficiency in the C8161 cells treated with a FAK antisense oligonucleosides and a 85% reduction in colony formation efficiency in the RD cells treated with FAK antisense compared to cells treated with missense

15   oligonucleosides. These results confirm that the loss of adhesion seen in cells after FAK antisense treatment is associated with reduced anchorage-independent growth.

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TABLE 4. ANCHORAGE-INDEPENDENT GROWTH OF FAK
ANTISENSE TREATED CELLS

C8161	Total # Colonies	Colony Forming Efficiency
MSN2	29,818 (±907)	59.6
FAK2AS	5,759 (±571)	11.5
RD	Total # Colonies	Colony Forming Efficiency
MSN2	10,205 (±2586)	20.4
FAK2AS	1544 (±630)	3.1

H.   **Inhibition of Tumor Formation in Nude Mice by FAK Antisense
Compounds**

The ability of FAK antisense compounds to inhibit tumor formation in
athymic nude mice was also tested. Four-week old female athymic nude mice
(Harlan Sprague-Dawley) were used in this study. Animals were maintained
under the guidelines of the National Institutes of Health and The University of
North Carolina School of Medicine. Mice were injected s.c. in the dorsolateral
left flank with cells (RD or C8161, 2 x 10^6) suspended in HBSS. Prior to
injection the cells were either treated with FAK antisense (FAK1AS or
FAK2AS) or missense control (MSN2) as previously described. Tumor growth
was monitored serially beginning several days after injection. Two perpendicular
measurements of the diameter of any palpable nodule were obtained, and an
estimated volume was calculated as $lw^2/2$. The animal was sacrificed at the end
of the experiment and examined for any intrusion of tumor through the body wall
or evidence of metastases to various body organs. The tumors were removed and
protein extracted for FAK analysis.

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As shown in FIG. 13, there was a significant lag time (almost 2 weeks) in the development of tumors using both RD and C8161 treated cells. This was surprising since related studies (see FIG. 4) had shown that such cells regain their FAK expression capability after seventy-two hours.

5   Inhibition of tumor growth was also tested *in vivo* by continuous delivery of antisense or missense oligonucleosides. Twelve nude mice were injected s.c. in the dorsolateral left flank with 2×10^6 C8161 cells and allowed to grow for 7 days until an approximately 1 cm lesion formed. Alzet 2002 osmotic pumps were surgically implanted s.c. over the right scapula of the mice and loaded to

10   provide 12.5 mg/kg/day of the oligonucleoside over a 14-day treatment period. Six mice were treated with antisense oligonucleoside; the other six mice were treated with a missense control oligonucleoside. Two of the mice died within the first 3 days and another died at 11 days, but evaluation of tumor volume at 14 days showed significant tumor inhibition in the antisense-treated animals,

15   compared to those treated with the missense control. The results of the *in vivo* studies are summarized in Table 5 below, for the surviving mice (5 surviving mice for the antisense oligonucleoside; 4 surviving mice for the missense oligonucleoside). No gross liver or pulmonary metastasis was observed. This was further confirmed in the case of pulmonary tissue by blinded, independent

20   histopathological examination of fixed lung tissue sections.

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TABLE 5. INHIBITION OF TUMOR GROWTH IN NUDE MICE USING FAK ANTISENSE COMPOUNDS

Antisense Tumor Volume (mm³)	Missense Tumor Volume (mm³)
125	750
125	350
100	500
63	650
125	
Mean 108 ± 27	Mean 563 ± 175

The foregoing results show that increased levels of p125^{FAK} are associated with tumor invasion and metastasis and that disruption of this pathway by attenuating p125^{FAK} expression with FAK antisense oligonucleosides significantly inhibits cellular adhesion, motility and anchorage independence. This data also points to FAK as a mediator of the processes which are downstream from other signaling molecules such as c-src and fyn.

Although the present invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that various changes and modifications may be made thereto, and various equivalents used, without departing from the spirit or scope of the claims. Therefore, the foregoing description should not be construed to limit the scope of the present invention, which is set forth in the appended claims.

40

SEQUENCE LISTING

(1) GENERAL INFORMATION:

    (i) APPLICANT: CANCE, William G.

                      LIU, Edison T.

                      OWENS, Lewis V.

    (ii) TITLE OF INVENTION: Oligonucleoside Compounds and Methods
                           for Inhibiting Tumor Growth, Invasion
                           and Metastasis

    (iii) NUMBER OF SEQUENCES: 8

    (iv) CORRESPONDENCE ADDRESS:

        (A) ADDRESSEE: Lyon & Lyon

        (B) STREET: 633 West Fifth Street, Suite 4700

        (C) CITY: Los Angeles

        (D) STATE: CA

        (E) COUNTRY: U.S.A.

        (F) ZIP: 90071-2066

    (v) COMPUTER READABLE FORM:

        (A) MEDIUM TYPE: Floppy disk

        (B) COMPUTER: IBM PC compatible

        (C) OPERATING SYSTEM: PC-DOS/MS-DOS

        (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

    (vi) CURRENT APPLICATION DATA:

        (A) APPLICATION NUMBER: US 08/276,843

        (B) FILING DATE: 18-JULY-1994

        (C) CLASSIFICATION: 03A1/0912

    (viii) ATTORNEY/AGENT INFORMATION:

        (A) NAME: Meier, Paul H.

        (B) REGISTRATION NUMBER: 32,274

        (C) REFERENCE/DOCKET NUMBER: 208/061

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        (A) TELEPHONE: 213/489-1600

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        (C) TELEX: 67-3510

(2) INFORMATION FOR SEQ ID NO:1:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 1052 amino acids

        (B) TYPE: amino acid

41

          (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: protein

     (iii) HYPOTHETICAL: No

     (iv) ANTI-SENSE: No

     (ix) FEATURE:

          (A) NAME/KEY: HUMFAK (human FAK)

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Met Ala Ala Ala Tyr Leu Asp Pro Asn Leu Asn His Thr Pro Asn Ser
1               5                   10                  15

Ser Thr Lys Thr His Leu Gly Thr Gly Met Glu Arg Ser Pro Gly Ala
            20                  25                  30

Met Glu Arg Val Leu Lys Val Phe His His Phe Glu Ser Ser Ser Glu
            35                  40                  45

Pro Thr Thr Trp Ala Ser Ile Ile Arg His Gly Asp Ala Thr Asp Val
        50                  55                  60

Arg Gly Ile Ile Gln Lys Ile Val Asp Ser His Lys Val Lys His Val
65                  70                  75                  80

Ala Cys Tyr Gly Phe Arg Leu Ser His Leu Arg Ser Glu Glu Val His
                85                  90                  95

Trp Leu His Val Asp Met Gly Val Ser Ser Val Arg Glu Lys Tyr Glu
            100                 105                 110

Leu Ala His Pro Pro Glu Glu Trp Lys Tyr Glu Leu Arg Ile Arg Tyr
            115                 120                 125

Leu Pro Lys Gly Phe Leu Asn Gln Phe Thr Glu Asp Lys Pro Thr Leu
    130                 135                 140

Asn Phe Phe Tyr Gln Gln Val Lys Ser Asp Tyr Met Gln Glu Ile Ala
145                 150                 155                 160

Asp Gln Val Asp Gln Glu Ile Ala Leu Lys Leu Gly Cys Leu Glu Ile
            165                 170                 175

Arg Arg Ser Tyr Trp Glu Met Arg Gly Asn Ala Leu Glu Lys Lys Ser
            180                 185                 190

Asn Tyr Glu Val Leu Glu Lys Asp Val Gly Leu Lys Arg Phe Phe Pro
        195                 200                 205

Lys Ser Leu Leu Asp Ser Val Lys Ala Lys Thr Leu Arg Lys Leu Ile
    210                 215                 220

Gln Gln Thr Phe Arg Gln Phe Ala Asn Leu Asn Arg Glu Glu Ser Ile
225                 230                 235                 240
```

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```
Leu Lys Phe Phe Glu Ile Leu Ser Pro Val Tyr Arg Phe Asp Lys Glu
              245                 250                 255
Cys Phe Lys Cys Ala Leu Gly Ser Ser Trp Ile Ile Ser Val Glu Leu
              260                 265                 270
Ala Ile Gly Pro Glu Glu Gly Ile Ser Tyr Leu Thr Asp Lys Gly Cys
              275                 280                 285
Asn Pro Thr His Leu Ala Asp Phe Thr Gln Val Gln Thr Ile Gln Tyr
          290             295                 300
Ser Asn Ser Glu Asp Lys Asp Arg Lys Gly Met Leu Gln Leu Lys Ile
305                 310                 315                 320
Ala Gly Ala Pro Glu Pro Leu Thr Val Thr Ala Pro Ser Leu Thr Ile
              325                 330                 335
Ala Glu Asn Met Ala Asp Leu Ile Asp Gly Tyr Cys Arg Leu Val Asn
              340                 345                 350
Gly Thr Ser Gln Ser Phe Ile Ile Arg Pro Gln Lys Glu Gly Glu Arg
              355                 360                 365
Ala Leu Pro Ser Ile Pro Lys Leu Ala Asn Ser Glu Lys Gln Gly Met
          370             375                 380
Arg Thr His Ala Val Ser Val Ser Glu Thr Asp Asp Tyr Ala Glu Ile
385                 390                 395                 400
Ile Asp Glu Glu Asp Thr Tyr Thr Met Pro Ser Thr Arg Asp Tyr Glu
              405                 410                 415
Ile Gln Arg Glu Arg Ile Glu Leu Gly Arg Cys Ile Gly Glu Gly Gln
              420                 425                 430
Phe Gly Asp Val His Gln Gly Ile Tyr Met Ser Pro Glu Asn Pro Ala
              435                 440                 445
Leu Ala Val Ala Ile Lys Thr Cys Lys Asn Cys Thr Ser Asp Ser Val
          450             455                 460
Arg Glu Lys Phe Leu Gln Glu Ala Leu Thr Met Arg Gln Phe Asp His
465                 470                 475                 480
Pro His Ile Val Lys Leu Ile Gly Val Ile Thr Glu Asn Pro Val Trp
              485                 490                 495
Ile Ile Met Glu Leu Cys Thr Leu Gly Glu Leu Arg Ser Phe Leu Gln
              500                 505                 510
Val Arg Lys Tyr Ser Leu Asp Leu Ala Ser Leu Ile Leu Tyr Ala Tyr
              515                 520                 525
```

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```
Gln Leu Ser Thr Ala Leu Ala Tyr Leu Glu Ser Lys Arg Phe Val His
        530                 535                 540

Arg Asp Ile Ala Ala Arg Asn Val Leu Val Ser Ser Asn Asp Cys Val
545                 550                 555                 560

Lys Leu Gly Asp Phe Gly Leu Ser Arg Tyr Met Glu Asp Ser Thr Tyr
                565                 570                 575

Tyr Lys Ala Ser Lys Gly Lys Leu Pro Ile Lys Trp Met Ala Pro Glu
                580                 585                 590

Ser Ile Asn Phe Arg Arg Phe Thr Ser Ala Ser Asp Val Trp Met Phe
                595                 600                 605

Gly Val Cys Met Trp Glu Ile Leu Met His Gly Val Lys Pro Phe Gln
        610                 615                 620

Gly Val Lys Asn Asn Asp Val Ile Gly Arg Ile Glu Asn Gly Glu Arg
625                 630                 635                 640

Leu Pro Met Pro Pro Asn Cys Pro Pro Thr Leu Tyr Ser Leu Met Thr
                645                 650                 655

Lys Cys Trp Ala Tyr Asp Pro Ser Arg Arg Pro Arg Phe Thr Glu Leu
                660                 665                 670

Lys Ala Gln Leu Ser Thr Ile Leu Glu Glu Glu Lys Ala Gln Gln Glu
        675                 680                 685

Glu Arg Met Arg Met Glu Ser Arg Arg Gln Ala Thr Val Ser Trp Asp
        690                 695                 700

Ser Gly Gly Ser Asp Glu Ala Pro Pro Lys Pro Ser Arg Pro Gly Tyr
705                 710                 715                 720

Pro Ser Pro Arg Ser Ser Glu Gly Phe Tyr Pro Ser Pro Gln His Met
                725                 730                 735

Val Gln Thr Asn His Tyr Gln Val Ser Gly Tyr Pro Gly Ser His Gly
                740                 745                 750

Ile Thr Ala Met Ala Gly Ser Ile Tyr Pro Gly Gln Ala Ser Leu Leu
                755                 760                 765

Asp Gln Thr Asp Ser Trp Asn His Arg Pro Gln Glu Ile Ala Met Trp
        770                 775                 780

Gln Pro Asn Val Glu Asp Ser Thr Val Leu Asp Leu Arg Gly Ile Gly
785                 790                 795                 800

Gln Val Leu Pro Thr His Leu Met Glu Glu Arg Leu Ile Arg Gln Gln
                805                 810                 815
```

44

Gln Glu Met Glu Glu Asp Gln Arg Trp Leu Glu Lys Glu Glu Arg Phe
            820   825   830

Leu Lys Pro Asp Val Arg Leu Ser Arg Gly Ser Ile Asp Arg Glu Asp
            835   840   845

Gly Ser Leu Gln Gly Pro Ile Gly Asn Gln His Ile Tyr Gln Pro Val
        850   855   860

Gly Lys Pro Asp Pro Ala Ala Pro Pro Lys Lys Pro Pro Arg Pro Gly
865   870   875   880

Ala Pro Gly His Leu Gly Ser Leu Ala Ser Leu Ser Ser Pro Ala Asp
                885   890   895

Ser Tyr Asn Glu Gly Val Lys Leu Gln Pro Gln Glu Ile Ser Pro Pro
            900   905   910

Pro Thr Ala Asn Leu Asp Arg Ser Asn Asp Lys Val Tyr Glu Asn Val
            915   920   925

Thr Gly Leu Val Lys Ala Val Ile Glu Met Ser Ser Lys Ile Gln Pro
            930   935   940

Ala Pro Pro Glu Glu Tyr Val Pro Met Val Lys Glu Val Gly Leu Ala
945   950   955   960

Leu Arg Thr Leu Leu Ala Thr Val Asp Glu Thr Ile Pro Leu Leu Pro
                965   970   975

Ala Ser Thr His Arg Glu Ile Glu Met Ala Gln Lys Leu Leu Asn Ser
            980   985   990

Asp Leu Gly Glu Leu Ile Asn Lys Met Lys Leu Ala Gln Gln Tyr Val
            995   1000   1005

Met Thr Ser Leu Gln Gln Glu Tyr Lys Lys Gln Met Leu Thr Ala Ala
            1010   1015   1020

His Ala Leu Ala Val Asp Ala Lys Asn Leu Leu Asp Val Ile Asp Gln
1025   1030   1035   1040

Ala Arg Leu Lys Met Leu Gly Gln Thr Arg Pro His
            1045   1050

(3) INFORMATION FOR SEQ ID NO:2:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 1052 amino acids

        (B) TYPE: amino acid

        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (iii) HYPOTHETICAL: No

45

(iv) ANTI-SENSE: No

(ix) FEATURE:

(A) NAME/KEY: MUSFAK (mouse FAK)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Ala Ala Ala Tyr Leu Asp Pro Asn Leu Asn His Thr Pro Ser Ser
1               5                   10                  15

Ser Thr Lys Thr His Leu Gly Thr Gly Met Glu Arg Ser Pro Gly Ala
            20                  25                  30

Met Glu Arg Val Leu Lys Val Phe His His Phe Glu Ser Ser Ser Glu
        35                  40                  45

Pro Thr Thr Trp Ala Ser Ile Ile Arg His Gly Asp Ala Thr Asp Val
        50                  55                  60

Arg Gly Ile Ile Gln Lys Ile Val Asp Ser His Lys Val Lys His Val
65                  70                  75                  80

Ala Cys Tyr Gly Phe Arg Leu Ser His Leu Arg Ser Glu Glu Val His
                85                  90                  95

Trp Leu His Val Asp Met Gly Val Ser Ser Val Arg Glu Lys Tyr Glu
            100                 105                 110

Leu Ala His Pro Pro Glu Glu Trp Lys Tyr Glu Leu Arg Ile Arg Tyr
            115                 120                 125

Leu Pro Lys Gly Phe Leu Asn Gln Phe Thr Glu Asp Lys Pro Thr Leu
        130                 135                 140

Asn Phe Phe Tyr Gln Gln Val Lys Ser Asp Tyr Met Gln Glu Ile Ala
145                 150                 155                 160

Asp Gln Val Asp Gln Glu Ile Ala Leu Lys Leu Gly Cys Leu Glu Ile
                165                 170                 175

Arg Arg Ser Tyr Trp Glu Met Arg Gly Asn Ala Leu Glu Lys Lys Ser
            180                 185                 190

Asn Tyr Glu Val Leu Glu Lys Asp Val Gly Leu Lys Arg Phe Phe Pro
            195                 200                 205

Lys Ser Leu Leu Asp Ser Val Lys Ala Lys Thr Leu Arg Lys Leu Ile
        210                 215                 220

Gln Gln Thr Phe Arg Gln Phe Ala Asn Leu Asn Arg Glu Glu Ser Ile
225                 230                 235                 240

Leu Lys Phe Phe Glu Ile Leu Ser Pro Val Tyr Arg Phe Asp Lys Glu
                245                 250                 255
```

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```
Cys Phe Lys Cys Ala Leu Gly Ser Ser Trp Ile Ile Ser Val Glu Leu
          260                 265                 270
Ala Ile Gly Pro Glu Glu Gly Ile Ser Tyr Leu Thr Asp Lys Gly Cys
          275                 280                 285
Asn Pro Thr His Leu Ala Asp Phe Asn Gln Val Gln Thr Ile Gln Tyr
          290                 295                 300
Ser Asn Ser Glu Asp Lys Asp Arg Lys Gly Met Leu Gln Leu Lys Ile
305                 310                 315                 320
Ala Gly Ala Pro Glu Pro Leu Thr Val Thr Ala Pro Ser Leu Thr Ile
                 325                 330                 335
Ala Glu Asn Met Ala Asp Leu Ile Asp Gly Tyr Cys Arg Leu Val Asn
          340                 345                 350
Gly Ala Thr Gln Ser Phe Ile Ile Arg Pro Gln Lys Glu Gly Glu Arg
          355                 360                 365
Ala Leu Pro Ser Ile Pro Lys Leu Ala Asn Ser Glu Lys Gln Gly Met
          370                 375                 380
Arg Thr His Ala Val Ser Val Ser Glu Thr Asp Asp Tyr Ala Glu Ile
385                 390                 395                 400
Ile Asp Glu Glu Asp Thr Tyr Thr Met Pro Ser Thr Arg Asp Tyr Glu
                 405                 410                 415
Ile Gln Arg Glu Arg Ile Glu Leu Gly Arg Cys Ile Gly Glu Gly Gln
                 420                 425                 430
Phe Gly Asp Val His Gln Gly Val Tyr Leu Ser Pro Glu Asn Pro Ala
          435                 440                 445
Leu Ala Val Ala Ile Lys Thr Cys Lys Asn Cys Thr Ser Asp Ser Val
          450                 455                 460
Arg Glu Lys Phe Leu Gln Glu Ala Leu Thr Met Arg Gln Phe Asp His
465                 470                 475                 480
Pro His Ile Val Lys Leu Ile Gly Val Ile Thr Glu Asn Pro Val Trp
                 485                 490                 495
Ile Ile Met Glu Leu Cys Thr Leu Gly Glu Leu Arg Ser Phe Leu Gln
          500                 505                 510
Val Arg Lys Tyr Ser Leu Asp Leu Ala Ser Leu Ile Leu Tyr Ala Tyr
          515                 520                 525
Gln Leu Ser Thr Ala Leu Ala Tyr Leu Glu Ser Lys Arg Phe Val His
          530                 535                 540
```

47

```
Arg Asp Ile Ala Ala Arg Asn Val Leu Val Ser Ser Asn Asp Cys Val
545                 550                 555                 560

Lys Leu Gly Asp Phe Gly Leu Ser Arg Tyr Met Glu Asp Ser Thr Tyr
                    565                 570                 575

Tyr Lys Ala Ser Lys Gly Lys Leu Pro Ile Lys Trp Met Ala Pro Glu
                580                 585                 590

Ser Ile Asn Phe Arg Arg Phe Thr Ser Ala Ser Asp Val Trp Met Phe
                595                 600                 605

Gly Val Cys Met Trp Glu Ile Leu Met His Gly Val Lys Pro Phe Gln
    610                 615                 620

Gly Val Lys Asn Asn Asp Val Ile Gly Arg Ile Glu Asn Gly Glu Arg
625                 630                 635                 640

Leu Pro Met Pro Pro Asn Cys Pro Pro Thr Leu Tyr Ser Leu Met Thr
                645                 650                 655

Lys Cys Trp Ala Tyr Asp Pro Ser Arg Arg Pro Arg Phe Thr Glu Leu
                660                 665                 670

Lys Ala Gln Leu Ser Thr Ile Leu Glu Glu Glu Lys Val Gln Gln Glu
                675                 680                 685

Glu Arg Met Arg Met Glu Ser Arg Arg Gln Ala Thr Val Ser Trp Asp
    690                 695                 700

Ser Gly Gly Ser Asp Glu Ala Pro Pro Lys Pro Ser Arg Pro Gly Tyr
705                 710                 715                 720

Pro Ser Pro Arg Ser Ser Glu Gly Phe Tyr Pro Ser Pro Gln His Met
                725                 730                 735

Val Gln Thr Asn His Tyr Gln Val Ser Gly Tyr Pro Gly Ser His Gly
                740                 745                 750

Ile Pro Ala Met Ala Gly Ser Ile Tyr Gln Gly Gln Ala Ser Leu Leu
                755                 760                 765

Asp Gln Thr Glu Leu Trp Asn His Arg Pro Gln Glu Met Ser Met Trp
    770                 775                 780

Gln Pro Ser Val Glu Asp Ser Ala Ala Leu Asp Leu Arg Gly Met Gly
785                 790                 795                 800

Gln Val Leu Pro Pro His Leu Met Glu Glu Arg Leu Ile Arg Gln Gln
                805                 810                 815

Gln Glu Met Glu Glu Asp Gln Arg Trp Leu Glu Lys Glu Glu Arg Phe
    820                 825                 830
```

48

```
Leu Lys Pro Asp Val Arg Leu Ser Arg Gly Ser Ile Asp Arg Glu Asp
        835             840             845
Gly Ser Phe Gln Gly Pro Thr Gly Asn Gln His Ile Tyr Gln Pro Val
        850             855             860
Gly Lys Pro Asp Pro Ala Ala Pro Pro Lys Lys Pro Pro Arg Pro Gly
865             870             875             880
Ala Pro Gly His Leu Ser Asn Leu Ser Ser Ile Ser Ser Pro Ala Asp
                885             890             895
Ser Tyr Asn Glu Gly Val Lys Leu Gln Pro Gln Glu Ile Ser Pro Pro
            900             905             910
Pro Thr Ala Asn Leu Asp Arg Ser Asn Asp Lys Val Tyr Glu Asn Val
        915             920             925
Thr Gly Leu Val Lys Ala Val Ile Glu Met Ser Ser Lys Ile Gln Pro
    930             935             940
Ala Pro Pro Glu Glu Tyr Val Pro Met Val Lys Glu Val Gly Leu Ala
945             950             955             960
Leu Arg Thr Leu Leu Ala Thr Val Asp Glu Thr Ile Pro Ala Leu Pro
            965             970             975
Ala Ser Thr His Arg Glu Ile Glu Met Ala Gln Lys Leu Leu Asn Ser
            980             985             990
Asp Leu Gly Glu Leu Ile Ser Lys Met Lys Leu Ala Gln Gln Tyr Val
        995             1000            1005
Met Thr Ser Leu Gln Gln Glu Tyr Lys Lys Gln Met Leu Thr Ala Ala
    1010            1015            1020
His Ala Leu Ala Val Asp Ala Lys Asn Leu Leu Asp Val Ile Asp Gln
1025            1030            1035            1040
Ala Arg Leu Lys Met Leu Gly Gln Thr Arg Pro His
            1045            1050
```

(4) INFORMATION FOR SEQ ID NO:3:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 1053 amino acids

        (B) TYPE: amino acid

        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

   (iii) HYPOTHETICAL: No

    (iv) ANTI-SENSE: No

    (ix) FEATURE:

49

(A) NAME/KEY: CHKFAK (chicken FAK)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Ala Ala Ala Tyr Leu Asp Pro Asn Leu Asn His Thr Pro Ser Ser
1   5   10   15

Ser Ala Lys Thr His Leu Gly Thr Gly Met Glu Arg Ser Pro Gly Ala
            20   25   30

Met Glu Arg Val Leu Lys Val Phe His Tyr Phe Glu Asn Ser Ser Glu
            35   40   45

Pro Thr Thr Trp Ala Ser Ile Ile Arg His Gly Asp Ala Thr Asp Val
            50   55   60

Arg Gly Ile Ile Gln Lys Ile Val Asp Cys His Lys Val Lys Asn Val
65   70   75   80

Ala Cys Tyr Gly Leu Arg Leu Ser His Leu Gln Ser Glu Glu Val His
            85   90   95

Trp Leu His Leu Asp Met Gly Val Ser Asn Val Arg Glu Lys Phe Glu
            100   105   110

Leu Ala His Pro Pro Glu Glu Trp Lys Tyr Glu Leu Arg Ile Arg Tyr
            115   120   125

Leu Pro Lys Gly Phe Leu Asn Gln Phe Thr Glu Asp Lys Pro Thr Leu
            130   135   140

Asn Phe Phe Tyr Gln Gln Val Lys Asn Asp Tyr Met Leu Glu Ile Ala
145   150   155   160

Asp Gln Val Asp Gln Glu Ile Ala Leu Lys Leu Gly Cys Leu Glu Ile
            165   170   175

Arg Arg Ser Tyr Gly Glu Met Arg Gly Asn Ala Leu Glu Lys Lys Ser
            180   185   190

Asn Tyr Glu Val Leu Glu Lys Asp Val Gly Leu Arg Arg Phe Phe Pro
            195   200   205

Lys Ser Leu Leu Asp Ser Val Lys Ala Lys Thr Leu Arg Lys Leu Ile
            210   215   220

Gln Gln Thr Phe Arg Gln Phe Ala Asn Leu Asn Arg Glu Glu Ser Ile
225   230   235   240

Leu Lys Phe Phe Glu Ile Leu Ser Pro Val Tyr Arg Phe Asp Lys Glu
            245   250   255

Cys Phe Lys Cys Ala Leu Gly Ser Ser Trp Ile Ile Ser Val Glu Leu
            260   265   270

50

```
Ala Ile Gly Pro Glu Glu Gly Ile Ser Tyr Leu Thr Asp Lys Gly Ala
          275                 280                 285
Asn Pro Thr His Leu Ala Asp Phe Asn Gln Val Gln Thr Ile Gln Tyr
          290                 295                 300
Ser Asn Ser Glu Asp Lys Asp Arg Lys Gly Met Leu Gln Leu Lys Ile
305                 310                 315                 320
Ala Gly Ala Pro Glu Pro Leu Thr Val Thr Ala Pro Ser Leu Thr Ile
                  325                 330                 335
Ala Glu Asn Met Ala Asp Leu Ile Asp Gly Tyr Cys Arg Leu Val Asn
              340                 345                 350
Gly Ala Thr Gln Ser Phe Ile Ile Arg Pro Gln Lys Glu Gly Glu Arg
          355                 360                 365
Ala Leu Pro Ser Ile Pro Lys Leu Ala Asn Asn Glu Lys Gln Gly Val
          370                 375                 380
Arg Ser His Thr Val Ser Val Ser Glu Thr Asp Asp Tyr Ala Glu Ile
385                 390                 395                 400
Ile Asp Glu Glu Asp Thr Tyr Thr Met Pro Ser Thr Arg Asp Tyr Glu
                  405                 410                 415
Ile Gln Arg Glu Arg Ile Glu Leu Gly Arg Cys Ile Gly Glu Gly Gln
              420                 425                 430
Phe Gly Asp Val His Gln Gly Ile Tyr Met Ser Pro Glu Asn Pro Ala
          435                 440                 445
Met Ala Val Ala Ile Lys Thr Cys Lys Asn Cys Thr Ser Asp Ser Val
          450                 455                 460
Arg Glu Lys Phe Leu Gln Glu Ala Leu Thr Met Arg Gln Phe Asp His
465                 470                 475                 480
Pro His Ile Val Lys Leu Ile Gly Val Ile Thr Glu Asn Pro Val Trp
                  485                 490                 495
Ile Ile Met Glu Leu Cys Thr Leu Gly Glu Leu Arg Ser Phe Leu Gln
              500                 505                 510
Val Arg Lys Phe Ser Leu Asp Leu Ala Ser Leu Ile Leu Tyr Ala Tyr
          515                 520                 525
Gln Leu Ser Thr Ala Leu Ala Tyr Leu Glu Ser Lys Arg Phe Val His
          530                 535                 540
Arg Asp Ile Ala Ala Arg Asn Val Leu Val Ser Ala Thr Asp Cys Val
545                 550                 555                 560
```

51

```
Lys Leu Gly Asp Phe Gly Leu Ser Arg Tyr Met Glu Asp Ser Thr Tyr
                565                 570                 575
Tyr Lys Ala Ser Lys Gly Lys Leu Pro Ile Lys Trp Met Ala Pro Glu
            580                 585                 590
Ser Ile Asn Phe Arg Arg Phe Thr Ser Ala Ser Asp Val Trp Met Phe
        595                 600                 605
Gly Val Cys Met Trp Glu Ile Leu Met His Gly Val Lys Pro Phe Gln
    610                 615                 620
Gly Val Lys Asn Asn Asp Val Ile Gly Arg Ile Glu Asn Gly Glu Arg
625                 630                 635                 640
Leu Pro Met Pro Pro Asn Cys Pro Pro Thr Leu Tyr Ser Leu Met Thr
                645                 650                 655
Lys Cys Trp Ala Tyr Asp Pro Ser Arg Arg Pro Arg Phe Thr Glu Leu
                660                 665                 670
Lys Ala Gln Leu Ser Thr Ile Leu Glu Glu Glu Lys Leu Gln Gln Glu
            675                 680                 685
Glu Arg Met Arg Met Glu Ser Arg Arg Gln Val Thr Val Ser Trp Asp
        690                 695                 700
Ser Gly Gly Ser Asp Glu Ala Pro Pro Lys Pro Ser Arg Pro Gly Tyr
705                 710                 715                 720
Pro Ser Pro Arg Ser Ser Glu Gly Phe Tyr Pro Ser Pro Gln His Met
                725                 730                 735
Val Gln Pro Asn His Tyr Gln Val Ser Gly Tyr Ser Gly Ser His Gly
            740                 745                 750
Ile Pro Ala Met Ala Gly Ser Ile Tyr Pro Gly Gln Ala Ser Leu Leu
            755                 760                 765
Asp Gln Thr Asp Ser Trp Asn His Arg Pro Gln Glu Val Ser Ala Trp
    770                 775                 780
Gln Pro Asn Met Glu Asp Ser Gly Thr Leu Asp Val Arg Gly Met Gly
785                 790                 795                 800
Gln Val Leu Pro Thr His Leu Met Glu Glu Arg Leu Ile Arg Gln Gln
            805                 810                 815
Gln Glu Met Glu Glu Asp Gln Arg Trp Leu Glu Lys Glu Glu Arg Phe
            820                 825                 830
Leu Val Met Lys Pro Asp Val Arg Leu Ser Arg Gly Ser Ile Glu Arg
            835                 840                 845
```

52

Glu Asp Gly Gly Leu Gln Gly Pro Ala Gly Asn Gln His Ile Tyr Gln
    850   855   860

Pro Val Gly Lys Pro Asp His Ala Ala Pro Pro Lys Lys Pro Pro Arg
865   870   875   880

Pro Gly Ala Pro His Leu Gly Ser Leu Ala Ser Leu Asn Ser Pro Val
                885   890   895

Asp Ser Tyr Asn Glu Gly Val Lys Ile Lys Pro Gln Glu Ile Ser Pro
            900   905   910

Pro Pro Thr Ala Asn Leu Asp Arg Ser Asn Asp Lys Val Tyr Glu Asn
        915   920   925

Val Thr Gly Leu Val Lys Ala Val Ile Glu Met Ser Ser Lys Ile Gln
    930   935   940

Pro Ala Pro Pro Glu Glu Tyr Val Pro Met Val Lys Glu Val Gly Leu
945   950   955   960

Ala Leu Arg Thr Leu Leu Ala Thr Val Asp Glu Ser Leu Pro Val Leu
            965   970   975

Pro Ala Ser Thr His Arg Glu Ile Glu Met Ala Gln Lys Leu Leu Asn
            980   985   990

Ser Asp Leu Ala Glu Leu Ile Asn Lys Met Lys Leu Ala Gln Gln Tyr
        995   1000   1005

Val Met Thr Ser Leu Gln Gln Glu Tyr Lys Lys Gln Met Leu Thr Ala
    1010   1015   1020

Ala His Ala Leu Ala Val Asp Ala Lys Asn Leu Leu Asp Val Ile Asp
1025   1030   1035   1040

Gln Ala Arg Leu Lys Met Ile Ser Gln Ser Arg Pro His
            1045   1050

(5) INFORMATION FOR SEQ ID NO:4:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 24 base pairs

        (B) TYPE: nucleic acid

        (C) STRANDEDNESS: single

        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid

    (iii) HYPOTHETICAL: No

    (iv) ANTI-SENSE: Yes

    (ix) FEATURE:

        (A) NAME/KEY: PAK1AS

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            (D) OTHER INFORMATION: inhibition of FAK expression

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

ACACTTGAAG CATTCCTTAT CAAA   24

(6) INFORMATION FOR SEQ ID NO:5:

        (i) SEQUENCE CHARACTERISTICS:

            (A) LENGTH: 20 base pairs

            (B) TYPE: nucleic acid

            (C) STRANDEDNESS: single

            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: other nucleic acid

        (iii) HYPOTHETICAL: No

        (iv) ANTI-SENSE: Yes

        (ix) FEATURE:

            (A) NAME/KEY: FAK2AS

            (D) OTHER INFORMATION: inhibition of FAK expression

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATAATCCAGC TTGAACCAAG   20

(7) INFORMATION FOR SEQ ID NO:6:

        (i) SEQUENCE CHARACTERISTICS:

            (A) LENGTH: 20 base pairs

            (B) TYPE: nucleic acid

            (C) STRANDEDNESS: single

            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: other nucleic acid

        (iii) HYPOTHETICAL: No

        (iv) ANTI-SENSE: Yes

        (ix) FEATURE:

            (A) NAME/KEY: MSN1

            (D) OTHER INFORMATION: 2-base mismatch control

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATAATCGAGC TTCAACCAAG   20

(8) INFORMATION FOR SEQ ID NO:7:

        (i) SEQUENCE CHARACTERISTICS:

            (A) LENGTH: 20 base pairs

            (B) TYPE: nucleic acid

            (C) STRANDEDNESS: single

            (D) TOPOLOGY: linear

54

        (ii) MOLECULE TYPE: other nucleic acid

       (iii) HYPOTHETICAL: No

        (iv) ANTI-SENSE: Yes

        (ix) FEATURE:

              (A) NAME/KEY: MSN2

              (D) OTHER INFORMATION: 5-base mismatch control

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATAATCGACG TTCAAGCAAG   20

(9) INFORMATION FOR SEQ ID NO:8:

         (i) SEQUENCE CHARACTERISTICS:

              (A) LENGTH: 20 base pairs

              (B) TYPE: nucleic acid

              (C) STRANDEDNESS: single

              (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: other nucleic acid

       (iii) HYPOTHETICAL: No

        (iv) ANTI-SENSE: Yes

        (ix) FEATURE:

              (A) NAME/KEY: WNT

              (D) OTHER INFORMATION: nonsense control sequence

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

AGCCCGAGCA GGTGGGGCTC   20

(10) INFORMATION FOR SEQ ID NO:9:

         (i) SEQUENCE CHARACTERISTICS:

              (A) LENGTH: 24 base pairs

              (B) TYPE: nucleic acid

              (C) STRANDEDNESS: single

              (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: other nucleic acid

       (iii) HYPOTHETICAL: No

        (iv) ANTI-SENSE: Yes

        (ix) FEATURE:

              (A) NAME/KEY: (G)4

              (D) OTHER INFORMATION: control sequence

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TATGCTGTGC CGGGGTCTTC GGGC   24

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What is claimed is:

1.   An oligonucleoside compound for inhibiting expression of a focal adhesion kinase protein comprising from about 6 to about 40 linked nucleosides in a sequence that is complementary to a target region of a focal adhesion kinase mRNA.

2.   The oligonucleoside compound of claim 1 wherein said focal adhesion kinase mRNA is a human mRNA.

3.   The oligonucleoside compound of claim 2 wherein said human focal adhesion kinase mRNA is an mRNA overexpressed in a transformed human cell.

4.   The oligonucleoside compound of claim 1 comprising from about 12 to about 30 linked nucleosides.

5.   The oligonucleoside compound of claim 1 wherein at least one of the internucleoside linkage structures in the compound is a non-phosphodiester linkage that is resistant to degradation by an endogenous cellular nuclease.

6.   The oligonucleoside compound of claim 5 wherein said at least one non-phosphodiester linkage is a linkage selected from the group consisting of phosphorothioate, phosphorodithioate, alkyl- or arylphosphonate, phosphoramidate, phosphotriester, alkyl- or arylphosphonothioate, aminoalkylphosphonate, aminoalkylphosphonothioate, phosphorofluoridate, boranophosphate, silyl, formacetal, thioformacetal, morpholino and peptide-based linkages.

7.   The oligonucleoside compound of claim 5 comprising at least one phosphorothioate linkage.

8.   The oligonucleoside compound of claim 7 comprising a plurality of phosphorothioate linkages.

9.   The oligonucleoside compound of claim 1 wherein said focal adhesion kinase mRNA target region is in a 5'-untranslated portion of the mRNA.

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10. The oligonucleoside compound of claim 1 wherein said focal adhesion kinase mRNA target region is in a coding portion of the mRNA.

11. The oligonucleoside compound of claim 2 wherein said complementary oligonucleoside sequence is specific for the target region of said human focal adhesion kinase mRNA, such that hybridization of the compound with unintended human nucleic acid sequences is minimized upon application of the compound to human cells.

12. A formulation comprising the oligonucleoside compound of any of claims 1, 2, 3 or 5 and a vehicle adapted to allow delivery of the compound to animal cells.

13. The formulation of claim 12 wherein said vehicle is a pharmaceutically acceptable carrier.

14. The formulation of claim 12 wherein said vehicle includes a material suitable to facilitate delivery of the oligonucleoside compound across a cell membrane of the animal subject.

15. The formulation of claim 14 wherein said material is a non-toxic lipid material.

16. A method of inhibiting growth of a transformed animal cell comprising administering to said animal cell the oligonucleoside compound of any of claims 1, 2, 3 or 5.

17. A method of inhibiting invasiveness of a transformed animal cell comprising administering to said animal cell the oligonucleoside compound of any of claims 1, 2, 3 or 5.

18. A method of inhibiting cell colony formation in transformed animal cells comprising administering to said animal cells the oligonucleoside compound of any of claims 1, 2, 3 or 5.

19. A method of inducing apoptosis of a transformed animal cell comprising administering to said animal cell the oligonucleoside compound of any of claims 1, 2, 3 or 5.

20. A method of reducing the rate of tumor formation attributable to transformed cells in an animal comprising administering to said animal the oligonucleoside compound of any of claims 1, 2, 3 or 5.

21. The method of claim 16 wherein said administration is to an animal subject in which transformed cells reside.

22. The method of claim 17 wherein said administration is to an animal subject in which transformed cells reside.

23. The method of claim 18 wherein said administration is to an animal subject in which transformed cells reside.

24. The method of claim 19 wherein said administration is to an animal subject in which transformed cells reside.

25. A method of treating cancer in a human comprising administering the oligonucleoside compound of any of claims 3, 4 or 5.

26. A method of treating cancer in a human comprising administering the formulation of claim 12.

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HUMFAK    1  MAAAYLDPNLNHTPNSSTKTHLGTGMERSPGAMERVLKVFHHFESSSEPTTWASIIRHGDATDVRGIIQKIVDSHKVKHV
MUSFAK    1  MAAAYLDPNLNHTPSSSTKTHLGTGMERSPGAMERVLKVFHHFESSSEPTTWASIIRHGDATDVRGIIQKIVDSHKVKHV
CHKFAK    1  MAAAYLDPNLNHTPSSSAKTHLGTGMERSPGAMERVLKVFHYFENSSEPTTWASIIRHGDATDVRGIIQKIVDCHKVKNV

HUMFAK   81  ACYGFRLSHLRSEEVHWLHVDMGVSSVREKYELAHPPEEWKYELRIRYLPKGFLNQFTEDKPTLNFFYQQVKSDYMQEIA
MUSFAK   81  ACYGFRLSHLRSEEVHWLHVDMGVSSVREKYELAHPPEEWKYELRIRYLPKGFLNQFTEDKPTLNFFYQQVKSDYMQEIA
CHKFAK   81  ACYGLRLSHLQSEEVHWLHLDMGVSNVREKFELAHPPEEWKYELRIRYLPKGFLNQFTEDKPTLNFFYQQVKNDYMLEIA

HUMFAK  161  DQVDQEIALKLGCLEIRRSYWEMRGNALEKKSNYEVLEKDVGLKRFFPKSLLDSVKAKTLRKLIQQTFRQFANLNREESI
MUSFAK  161  DQVDQEIALKLGCLEIRRSYWEMRGNALEKKSNYEVLEKDVGLKRFFPKSLLDSVKAKTLRKLIQQTFRQFANLNREESI
CHKFAK  161  DQVDQEIALKLGCLEIRRSYGEMRGNALEKKSNYEVLEKDVGLRRFFPKSLLDSVKAKTLRKLIQQTFRQFANLNREESI

                          recombinant peptide
                 FAK1AS   FAK2AS
HUMFAK  241  LKFFEILSPVYRFDKECFKCALGSSWIISVELAIGPEEGISYLTDKGCNPTHLADFTQVQTIQYSNSEDKDRKGMLQLKI
MUSFAK  241  LKFFEILSPVYRFDKECFKCALGSSWIISVELAIGPEEGISYLTDKGCNPTHLADFNQVQTIQYSNSEDKDRKGMLQLKI
CHKFAK  241  LKFFEILSPVYRFDKECFKCALGSSWIISVELAIGPEEGISYLTDKGANPTHLADFNQVQTIQYSNSEDKDRKGMLQLKI

HUMFAK  321  AGAPEPLTVTAPSLTIAENMADLIDGYCRLVNGTSQSFIIRPQKEGEGERALPSIPKLANSEKQGMRTHAVSVSETDDYAEI
MUSFAK  321  AGAPEPLTVTAPSLTIAENMADLIDGYCRLVNGATQSFIIRPQKEGEGERALPSIPKLANSEKQGMRTHAVSVSETDDYAEI
CHKFAK  321  AGAPEPLTVTAPSLTIAENMADLIDGYCRLVNGATQSFIIRPQKEGEGERALPSIPKLANNEKQGVRSHTVSVSETDDYAEI

                          kinase domain
HUMFAK  401  IDEEDTYTMPSTRDYEIQRERIE  LGRCIGEGQFGDVHQGIYMSPENPALAVAIKTCKNCTSDSVREKFLQEALTMRQFDH
MUSFAK  401  IDEEDTYTMPSTRDYEIQRERIE  LGRCIGEGQFGDVHQGVYLSPENPALAVAIKTCKNCTSDSVREKFLQEALTMRQFDH
CHKFAK  401  IDEEDTYTMPSTRDYEIQRERIE  LGRCIGEGQFGDVHQGIYMSPENPAMAVAIKTCKNCTSDSVREKFLQEALTMRQFDH

HUMFAK  481  PHIVKLIGVITENPVWIIMELCTLGELRSFLQVRKYSLDLASLILYAYQLSTALAYLESKRFVHRDIAARNVLVSSNDCV
MUSFAK  481  PHIVKLIGVITENPVWIIMELCTLGELRSFLQVRKYSLDLASLILYAYQLSTALAYLESKRFVHRDIAARNVLVSSNDCV
CHKFAK  481  PHIVKLIGVITENPVWIIMELCTLGELRSFLQVRKFSLDLASLILYAYQLSTALAYLESKRFVHRDIAARNVLVSATDCV

HUMFAK  561  KLGDFGLSRYMEDSTYYKASKGKLPIKWMAPESINFRRFTSASDVWMFGVCMWEILMHGVK  PFQGVKNNDVIGRIENGER
MUSFAK  561  KLGDFGLSRYMEDSTYYKASKGKLPIKWMAPESINFRRFTSASDVWMFGVCMWEILMHGVK  PFQGVKNNDVIGRIENGER
CHKFAK  561  KLGDFGLSRYMEDSTYYKASKGKLPIKWMAPESINFRRFTSASDVWMFGVCMWEILMHGVK  PFQGVKNNDVIGRIENGER
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FIG. 1A

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HUMFAK   641  LPMPPNCPPTLYSLMTKCWAYDPSRRPRFTELKAQLSTILEEEKAQQEERMRMESRRQATVSWDSGGSDEAPPKPSRPGY
MUSFAK   641  LPMPPNCPPTLYSLMTKCWAYDPSRRPRFTELKAQLSTILEEEKVQQEERMRMESRRQATVSWDSGGSDEAPPKPSRPGY
CHKFAK   641  LPMPPNCPPTLYSLMTKCWAYDPSRRPRFTELKAQLSTILEEEKLQQEERMRMESRRQVTVSWDSGGSDEAPPKPSRPGY

HUMFAK   721  PSPRSSEGFYPSPQHMVQTNHYQVSGYPGSHGITAMAGSIYPGQASLLDQTDSWNHRPQEIAMWQPNVEDSTVLDLRGIG
MUSFAK   721  PSPRSSEGFYPSPQHMVQTNHYQVSGYPGSHGIPAMAGSIYQGQASLLDQTELWNHRPQEMSMWQPSVEDSAALDLRGMG
CHKFAK   721  PSPRSSEGFYPSPQHMVQPNHYQVSGYSGSHGIPAMAGSIYPGQASLLDQTDSWNHRPQEVSAWQPNMEDSGTLDVRGMG

HUMFAK   801  QVLPTHLMEERLIRQQQEMEEDQRWLEKEERFL..KPDVRLSRGSIDREDGSLQGPIGNQHIYQPVGKPDPAAPPKKPPR
MUSFAK   801  QVLPPHLMEERLIRQQQEMEEDQRWLEKEERFL..KPDVRLSRGSIDREDGSFQGPTGNQHIYQPVGKPDPAAPPKKPPR
CHKFAK   801  QVLPTHLMEERLIRQQQEMEEDQRWLEKEERFLVMKPDVRLSRGSIEREDGGLQGPAGNQHIYQPVGKPDHAAPPKKPPR

HUMFAK   879  PGAPGHLGSLASLSSPADSYNEGVKLQPQEISPPPTANLDRSNDKVYENVTGLVKAVIEMSSKIQPAPPEEYVPMVKEVG
MUSFAK   879  PGAPGHLSNLSSISSPADSYNEGVKLQPQEISPPPTANLDRSNDKVYENVTGLVKAVIEMSSKIQPAPPEEYVPMVKEVG
CHKFAK   881  PGAP.HLGSLASLNSPVDSYNEGVKIKPQEISPPPTANLDRSNDKVYENVTGLVKAVIEMSSKIQPAPPEEYVPMVKEVG

HUMFAK   959  LALRTLLATVDETIPLLPASTHREIEMAQKLLNSDLGELINKMKLAQQYVMTSLQQEYKKQMLTAAHALAVDAKNLLDVI
MUSFAK   959  LALRTLLATVDETIPALPASTHREIEMAQKLLNSDLGELISKMKLAQQYVMTSLQQEYKKQMLTAAHALAVDAKNLLDVI
CHKFAK   960  LALRTLLATVDESLPVLPASTHREIEMAQKLLNSDLAELINKMKLAQQYVMTSLQQEYKKQMLTAAHALAVDAKNLLDVI

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CHKFAK  1040  DQARLKMISQSRPH
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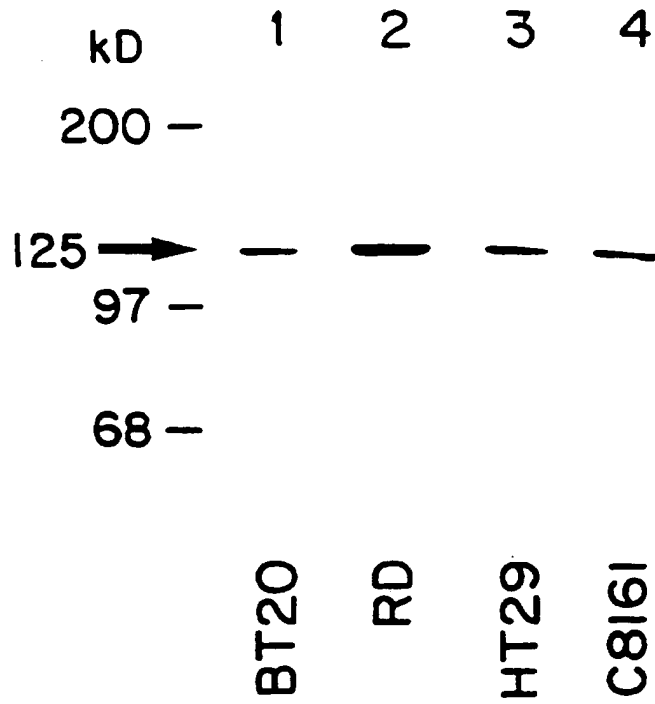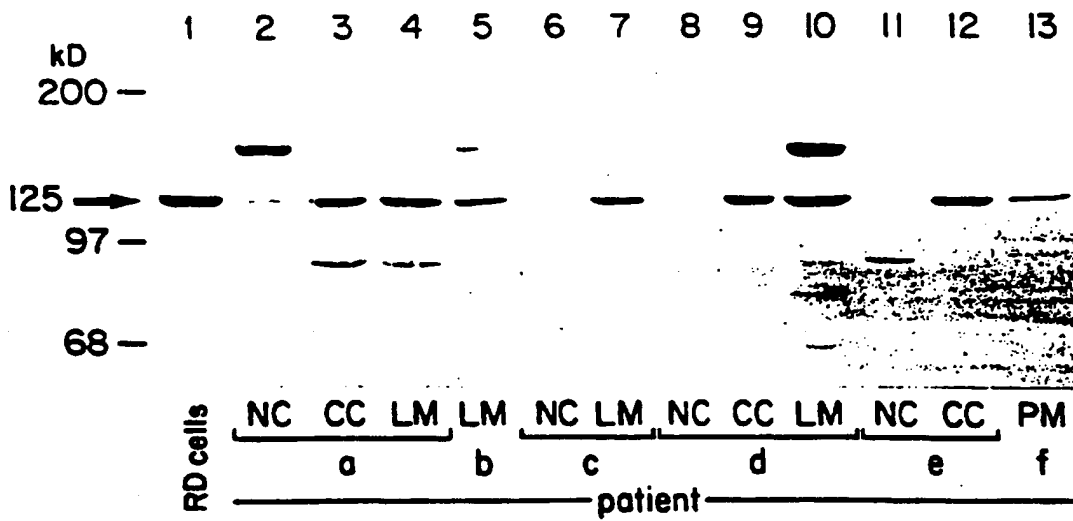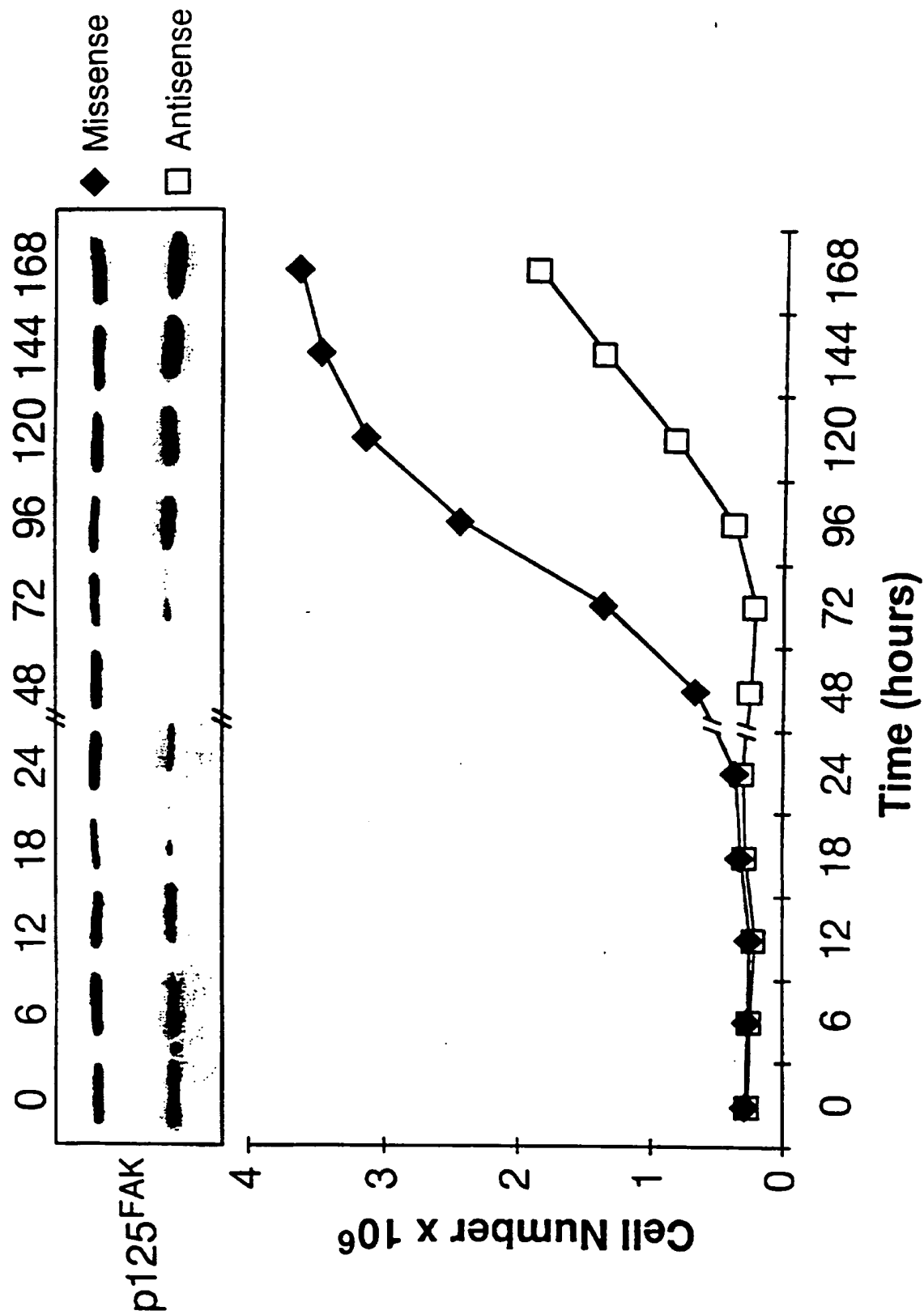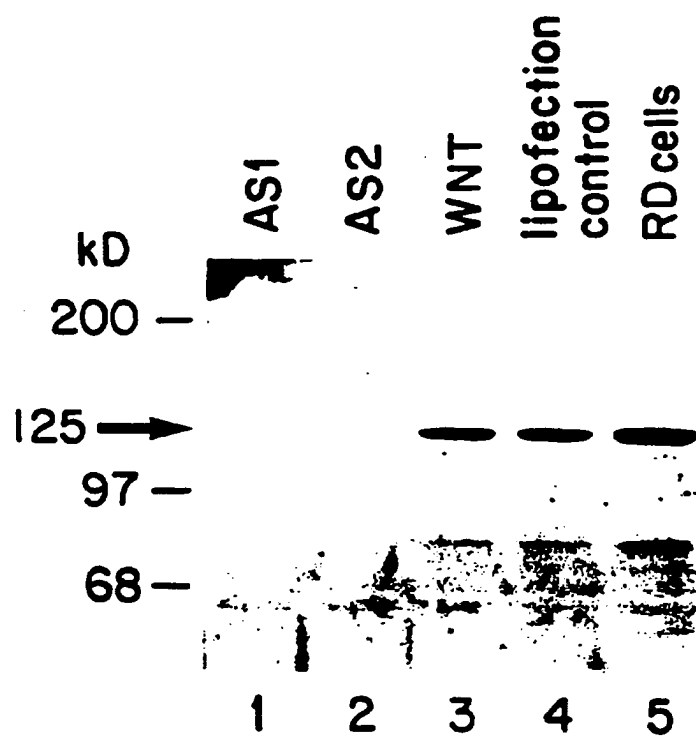
FIG. 1B

FIG. 2

FIG. 3

FIG. 4

FIG. 5
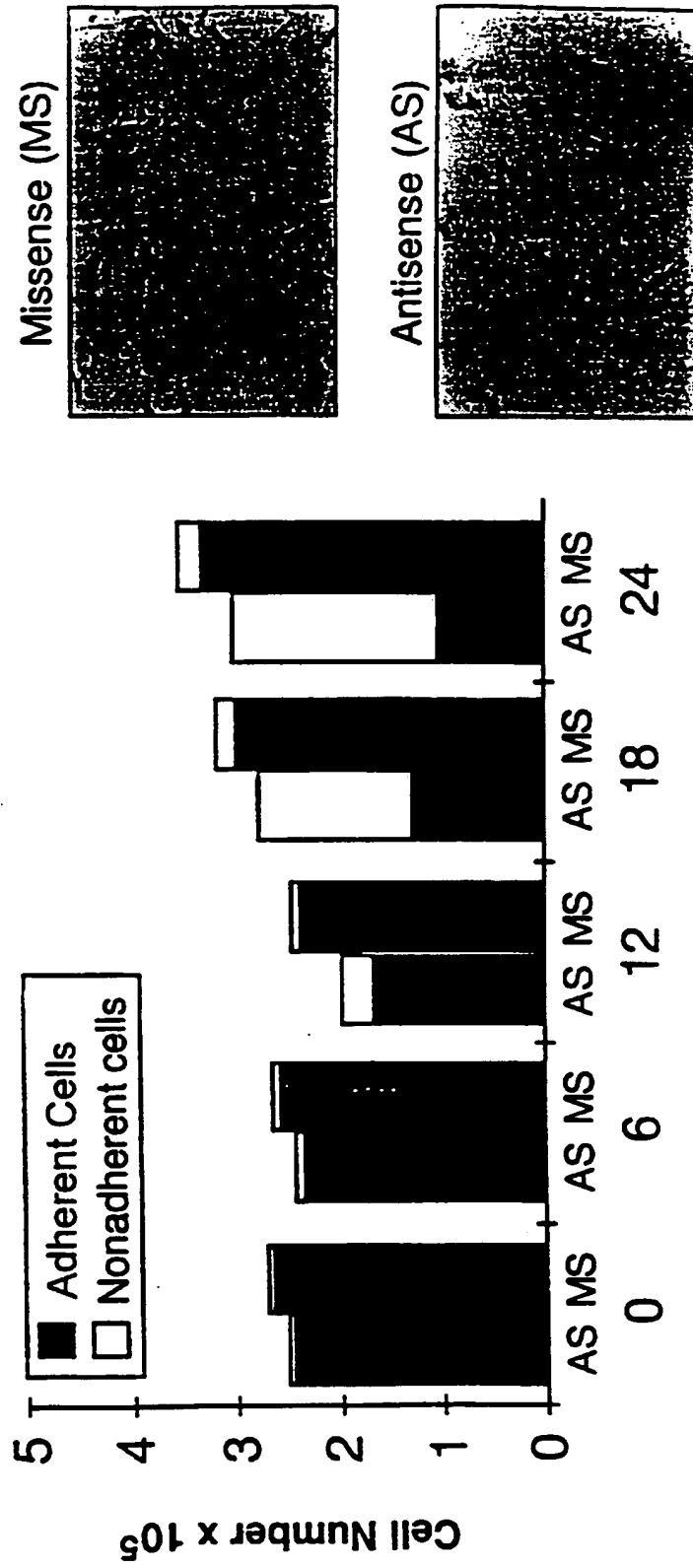
FIG. 6A
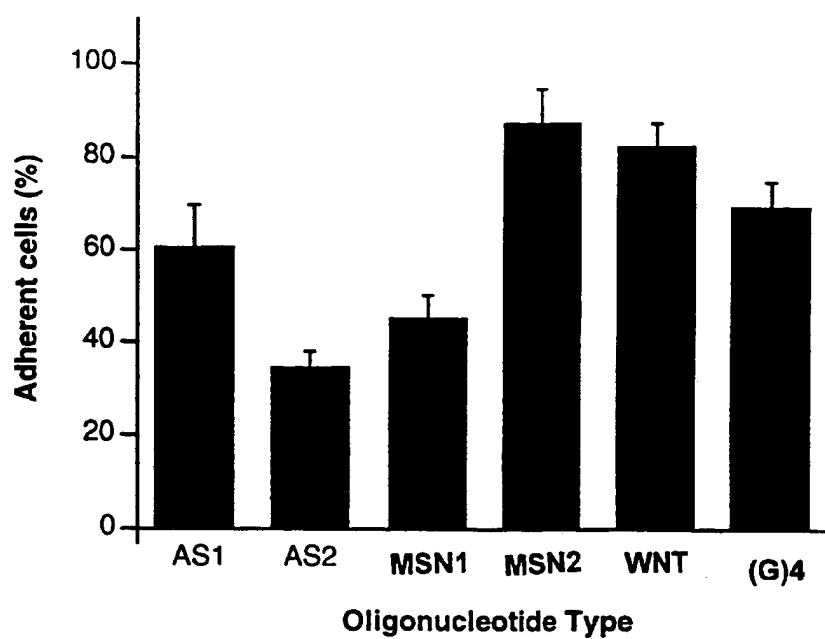
FIG. 6B
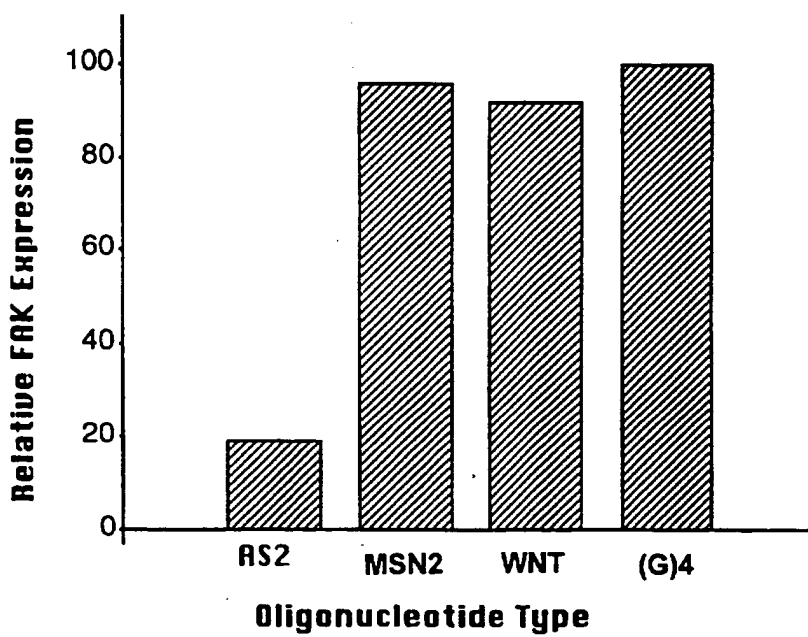
FIG. 6C
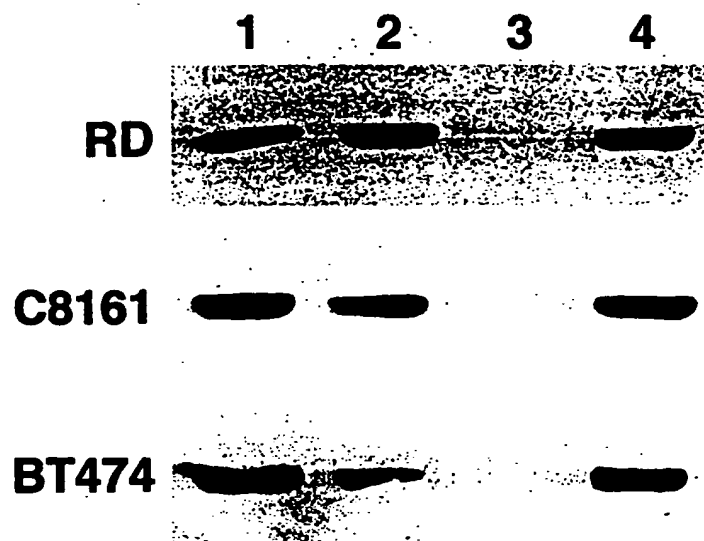
FIG. 7
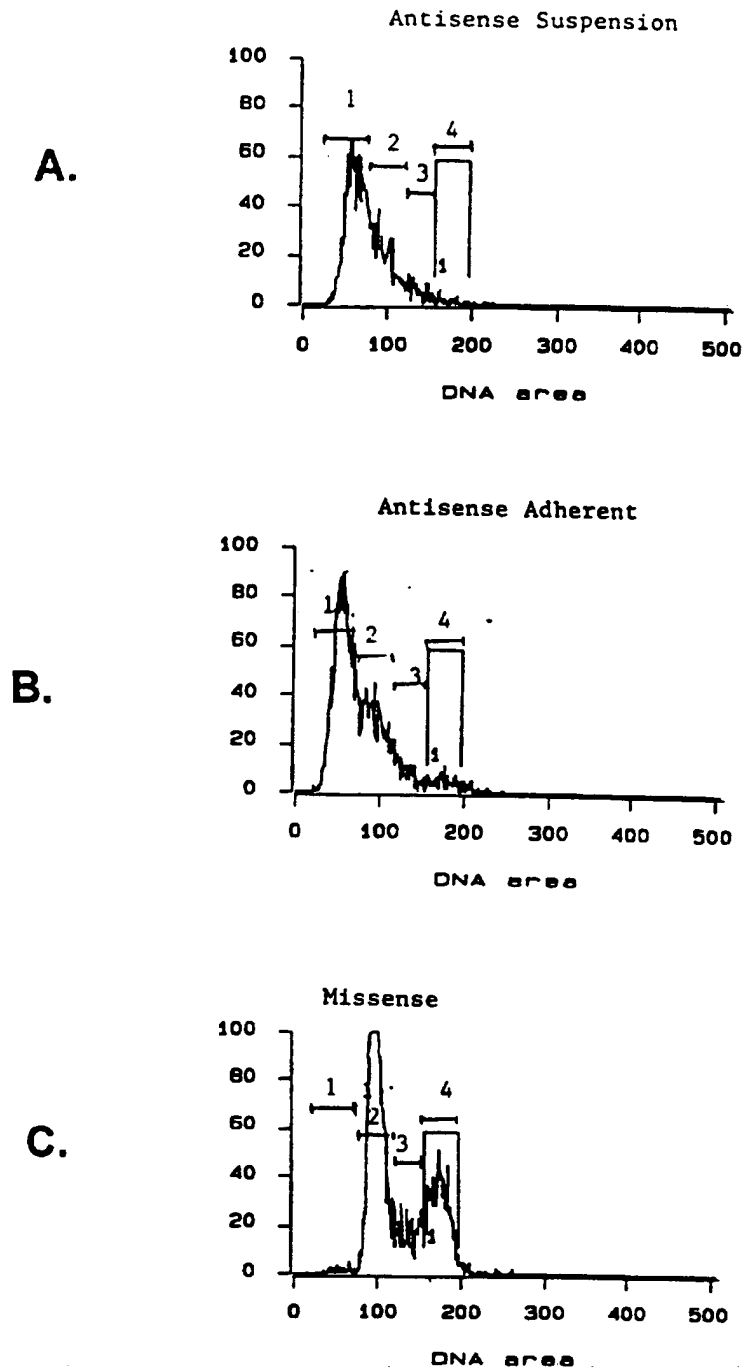
FIG. 8
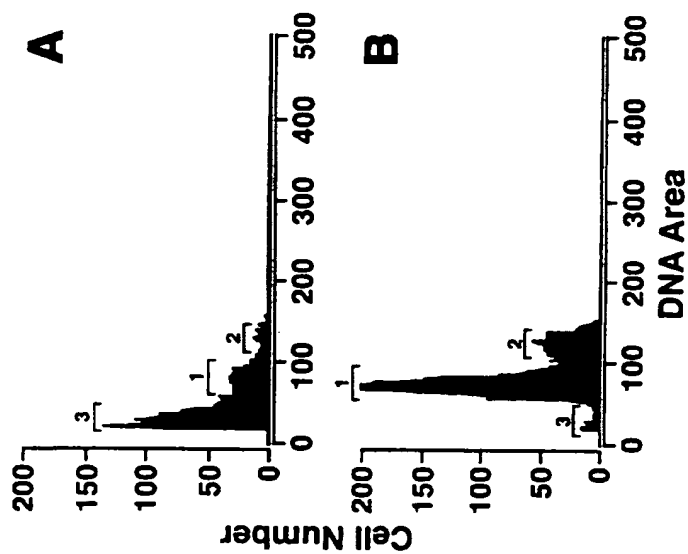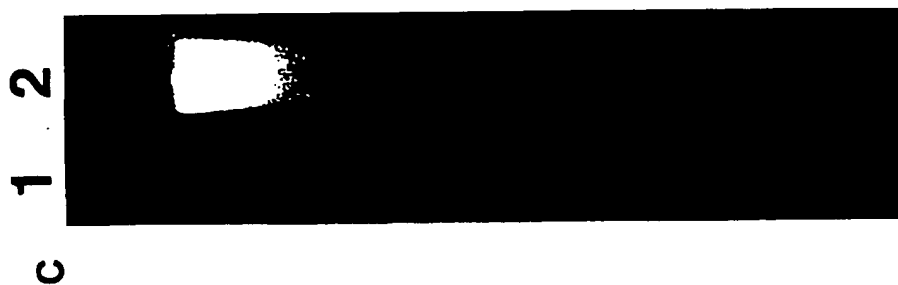
FIG. 9
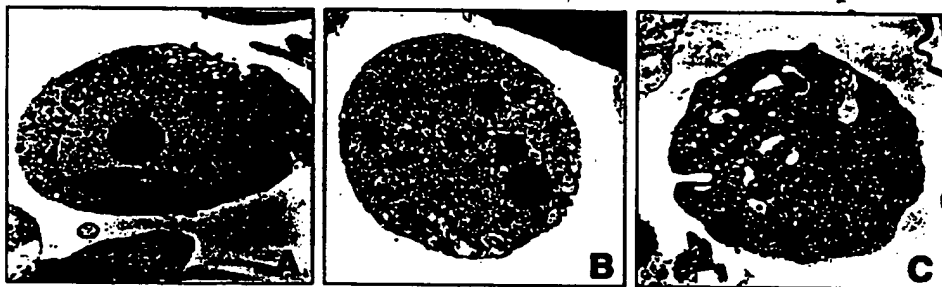
FIG. 10
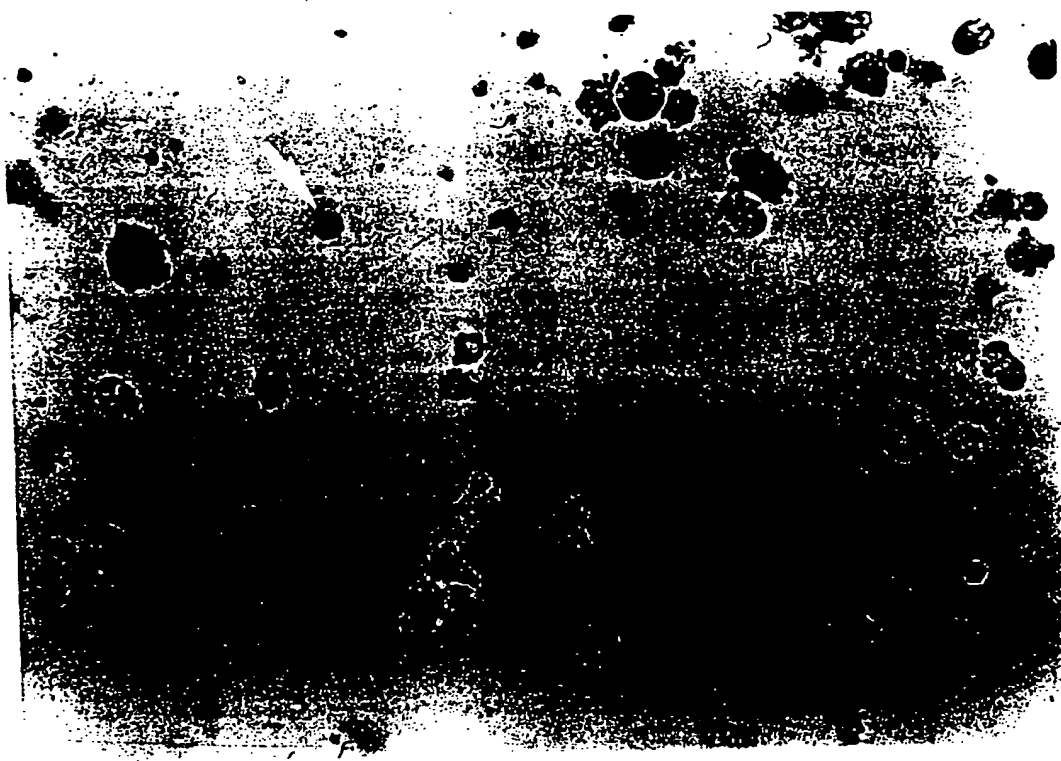
FIG. 11
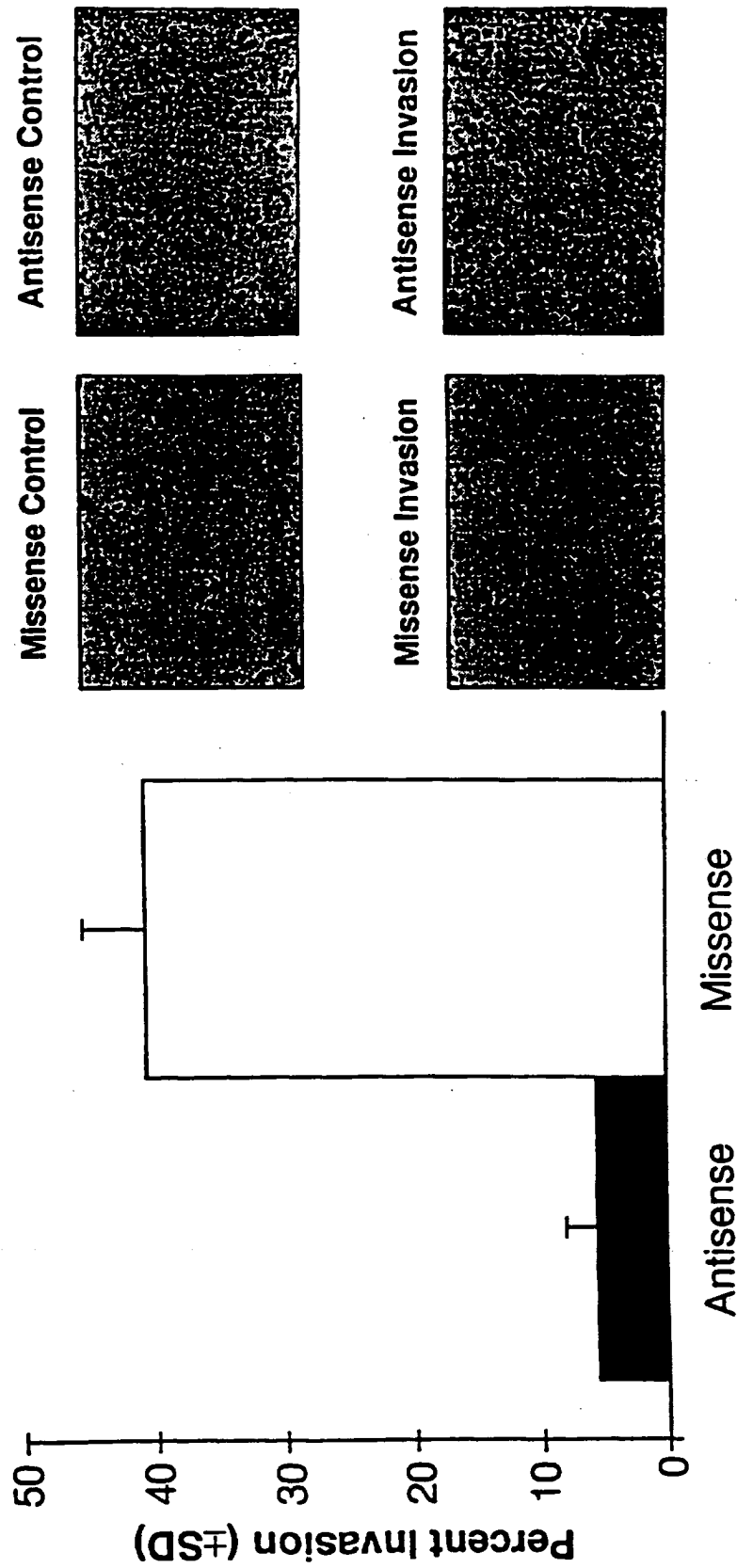
FIG. 12

2.0 x 10⁵ C8161 Cells Plated on a Boyden Chamber/Matrigel Interface

FIG. 13
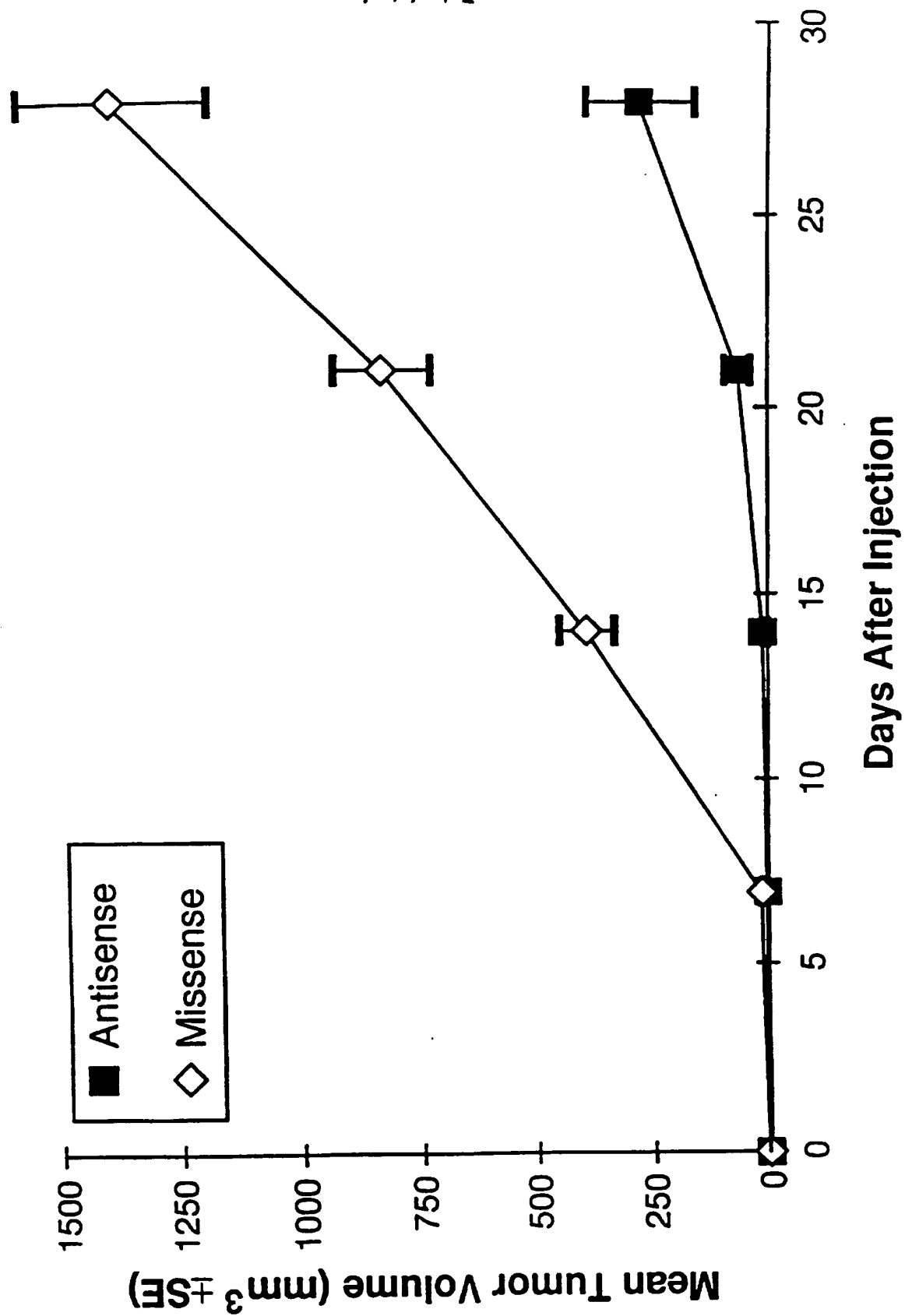
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07H 21/04
US CL : 536/24.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/24.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	American Journal of Physiology, Volume 9, Number 1, issued July 1993, NECKERS ET AL., "Antisense technology: biological utility and practical considerations", pages L1-L12, see pages L3-L8	1-26
Y	Biochemical and Biophysical Research Communications, Volume 190, issued 15 January 1993, ANDRE ET AL., "Expression of an n-terminally truncated form of human focal adhesion kinase in brain", pages 140-147, see page 144	1-26

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 OCTOBER 1995	01 NOV 1995

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	JOHN S. BRUSCA
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Form PCT/ISA/210 (second sheet)(July 1992)*

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Proc. Natl. Acad. Sci. U.S.A., Volume 89, issued June 1992, SCHALLER ET AL., "pp125FAK, a structurally distinctive protein-tyrosine kinase associated with focal adhesions", pages 5192-5196, see page 5192	1-26
Y	Molecular Biology of the Cell, Volume 5, issued April 1994, XING ET AL., "Direct interaction of v-Src with the focal adhesion kinase mediated by the Src SH2 domain", pages 413-421, see page 413	1-26
Y	DNA and Cell Biology, Volume 12, Number 9, issued November 1993, WHITNEY ET AL., "Human T and B lymphocytes express a structurally conserved focal adhesion kinase, pp125FAK", pages 823-827, see pages 825-827	1-26
Y	Journal of Cell Biology, Volume 119, Number 4, issued November 1992, LIPFERT ET AL., "Integrin-dependent phosphorylation and activation of the protein tyrosine kinase pp125FAK in platelets", pages 905-912, see page 905	1-26
Y	Journal of Biological Chemistry, Volume 267, Number 27, issued September 1992, ZACHARY ET AL., "Bombesin, vasopressin, and endothelin stimulation of tyrosine phosphorylation in swiss 3T3 cells", pages 19031-19034, see page 19031	1-26

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